HEREDITY

An International Journal of Genetics

HEREDITY

An International Journal of Genetics

Edited by

Kenneth Mather CBE FRS
Birmingham

David Catcheside FRS
Birmingham

John Thoday
Sheffield

Cyril Darlington FRS
Oxford

Sir Ronald Fisher FRS
Cambridge

Volume 13
1959

Oliver and Boyd
London and Edinburgh

Medical Library
610.5
H542
V.13

PRINTED IN GREAT BRITAIN BY
OLIVER AND BOYD LTD., EDINBURGH

Transfer to:
Medical
1-17-63

CONTENTS

PART I. FEBRUARY

PAGE

PATEMAN, J. A. (Sheffield and Melbourne). The effect of selection on ascospore size in *Neurospora crassa* 1

LYON, MARY F., and PHILLIPS, RITA J. S. (Harwell). Crossing-over in mice heterozygous for *t*-alleles 23

BARBER, H. N. (Tasmania). Physiological genetics of *Pisum II*. Photoperiodism and vernalisation 33

STOREY, H. H., and HOWLAND, AUDRIE K. (East Africa). Resistance in maize to the tropical American rust fungus, *Puccinia polysora*. II. Linkage of genes **Rpp**$_1$ and **Rpp**$_2$ 61

FORBES, E. (Glasgow). Use of mitotic segregation for assigning genes to linkage groups in *Aspergillus nidulans* 67

DAY, P. R. (Bayfordbury). A cytoplasmically controlled abnormality in the tetrads of *Coprinus lagopus* 81

DARLINGTON, C. D., and SHAW, G. W. (Oxford). Parallel polymorphism in the heterochromatin of *Trillium* species 89

NOTES AND COMMENTS :

VAN DER VEEN, J. H. The 2 × 2 genotype-environment table . . 123

CROSBY, JACK L. Outcrossing on homostyle primroses . . . 127

REVIEWS :

WADDINGTON, C. H. The strategy of the genes 133

WILLIAMS, GLANVILLE. The sanctity of life and the criminal law . . 136

MÜNTZING, ARNE, Vererbungslehre 138

RIEGER, R., and MICHAELIS, A. Genetisches und Cytogenetisches Wörterbuch 139

MARQUARDT, HANS. Natürliche und Künstliche Erbänderungen . . 139

DARLINGTON, C. D. Evolution of genetic systems 139

HERSKOWITZ, I. H. Bibliography on the genetics of *Drosophila*. Part 3 . 140

GENETICAL SOCIETY OF GREAT BRITAIN : Abstracts of Papers . . . 141

PART 2. MAY

BODMER, W. F., and PARSONS, P. A. (Cambridge). The analogy between factorial experimentation and balanced multi-point linkage tests . . 145

BODMER, W. F. (Cambridge). Multiplicative effects and the logarithmic transformation in the analysis of balanced multi-point linkage tests . 157

ROBINSON, R. (London). Genetic studies of the Syrian hamster. II. Partial albinism 165

PAGE

FISHER, R. A. (Cambridge). An algebraically exact examination of junction formation and transmission in parent-offspring inbreeding . . . 179

THODAY, J. M. (Sheffield). Effects of disruptive selection. I. Genetic flexibility 187

THODAY, J. M., and BOAM, T. B. (Sheffield). Effects of disruptive selection. II. Polymorphism and divergence without isolation 205

NOLTE, D. J. (Johannesburg). The eye-pigmentary system of *Drosophila*. VII. The white locus 219

NOLTE, D. J. (Johannesburg). The eye-pigmentary system of *Drosophila*. VIII. Series of multiple alleles 233

LEVITAN, M., and SALZANO, F. M. (Philadelphia and Brazil). Studies of linkage in populations. III. Non-random association of linked inversions 243

SAUNDERS, J. H. (Sudan). Studies in wild species of cotton. I. Variation within *Gossypium anomalum* 249

NOTES AND COMMENTS :

WILLIAMSON, M. H. Differential damage in a mixed colony of the land snails *Cepæa nemoralis* and *C. hortensis* 261

WILSON, J. Y. Duration of meiosis in relation to temperature . . 263

REVIEWS :

HUTT, F. B. Genetic resistance to disease in domestic animals . . 269

ELLIOTT, F. C. Plant breeding and cytogenetics 269

ANDERSON, V. E. Variables related to human breast cancer . . 270

SANKEY, JOHN. A guide to field biology 271

PART 3. AUGUST

NOLTE, D. J. (Johannesburg). The eye-pigmentary system of *Drosophila*. IX. Heterozygous effects of eye-colour genes 273

RENNER, O. (München). Somatic conversion in the heredity of the *cruciata* character in *Œnothera* 283

MANDEL, S. P. H. (Cambridge). The stability of a multiple allelic system . 289

GREEN, M. M. (Davis). Spatial and functional properties of pseudoalleles at the *white* locus in *Drosophila melanogaster* 303

COOPER, J. P. (Aberystwyth). Selection and population structure in *Lolium*. I. The initial populations 317

LYON, MARY F. (Harwell). Some evidence concerning the " mutational load " in inbred strains of mice 341

FORMAN, B., FORD, E. B., and MCWHIRTER, K. C. (Aberdeen and Oxford). An evolutionary study of the butterfly *Maniola jurtina* in the North of Scotland 353

CREED, E. R., DOWDESWELL, W. H., FORD, E. B., and MCWHIRTER, K. G. (Oxford). Evolutionary studies on *Maniola jurtina*: the English mainland, 1956-57 363

PAGE

PARSONS, P. A. (Cambridge). Dependence of genotypic viabilities on co-existing genotypes in *Drosophila* 393

NOTES AND COMMENTS :

CATCHESIDE, D. G. Segregation in tetraploid potatoes 403

REVIEW :

LERNER, I. M. The genetical basis of selection 409

GENETICAL SOCIETY OF GREAT BRITAIN : Abstracts of Papers 411

PART 4. NOVEMBER

TODD, G. F., and MASON, J. I. (London). Concordance of smoking habits in monozygotic and dizygotic twins 417

COOPER, J. P. (Aberystwyth). Selection and population structure in *Lolium*. II. Genetic control of date of ear emergence 445

COOPER, J. P. (Aberystwyth). Selection and population structure in *Lolium*. III. Selection for date of ear emergence 461

O'DONALD, P., and DAVIS, P. E. (Cambridge and Fair Isle). The genetics of the colour phases of the Arctic Skua 481

BODMER, W. F., and PARSONS, P. A. (Cambridge). Factorial analysis of balanced four- and higher-point linkage tests 487

ROBERTS, C., GANESAN, A. T., and HAUPT, W. (Copenhagen). Genetics of melibiose fermentation in *Saccharomyces italicus* var. *melibiosi* 499

NOTES AND COMMENTS :

SCHEUER, P. A. G., and MANDEL, S. P. H. An inequality in population genetics 519

JINKS, J. L. The genetical basis of " duality " in *imperfect* fungi . . 525

REVIEWS :

BONNER, J. T. The evolution of development 529

HUXLEY, J. S. Biological aspects of cancer 529

MAYR, E. (ed.). The species problem 530

BUTLER, J. A. V. Inside the living cell 530

HAYASHI, T. (ed.). Subcellular particles 531

FRASER ROBERTS, J. A. An introduction to medical genetics . . 531

ZORN, W. *et al.* Tierzüchtungslehre 533

ARNOLD, H. Vaganten, Komödianten, Fieranten und Briganten . . 533

EVERSLEY, D. E. C. Social theories of fertility and the Malthusian debate 537

CHESSER, E. An outline of human relationships 537

BOOKS RECEIVED 538

INDEX 539

HEREDITY VOLUME 13 PART 1 FEBRUARY 1959

THE EFFECT OF SELECTION ON ASCOSPORE SIZE IN *NEUROSPORA CRASSA*

J. A. PATEMAN
Department of Genetics, University of Sheffield *

Received 18.iii.58

1. INTRODUCTION

THE heterothallic fungus *Neurospora crassa* develops, under appropriate conditions, asci each of which contains eight ascospores in a linear array. When isolated from the wild and maintained under the usual laboratory conditions, the number of mature ascospores per ascus is always eight and the size of the normal ascospores varies very little. However, large ascospores, which are approximately twice the normal size, occasionally occur. Their frequency is less than one in ten thousand. Cultures from a number of these large ascospores were used as the starting point in an experiment in which selection was exercised for large ascospore size. The results of the selection experiment and of other studies on the inheritance of ascospore size in *Neurospora crassa* are reported in this paper.

2. METHODS

The strains used have complicated pedigrees but all, with the possible exception of the St Lawrence wild-type, were derived originally from the Abbot and/or the Lindegren wild-types. The wild-type, St Lawrence *A* (StL. *A*), which was obtained from Dr D. Newmeyer, is probably also derived to a large extent from the Abbot and Lindegren wild-types.

The vegetative cultures were maintained on agar slopes of Fries No. 3 (Beadle and Tatum, 1945) medium. All crosses were made on agar slopes of a medium favouring sexual reproduction (Westergaard and Mitchell, 1947) and incubated at 25° C.

The ascospores were measured with a micrometer eyepiece and the unit of measurement is approximately 2·17 μ.

3. SELECTION FOR INCREASED ASCOSPORE SIZE

(i) *Method of selection*

A particular cross between two apparently normal strains made in this laboratory produced an abnormally high frequency, approximately 3·4 per cent. of large (giant) ascospores. Four of these large ascospores germinated and gave good cultures and two fertile crosses were obtained from intercrosses between them. The frequency of large ascospores increased significantly (to about 11·4 per cent.) in

* Present address : Department of Botany, University of Melbourne, Australia.

these crosses, and it was therefore decided to begin a simple selection experiment.

The following procedure was adopted. All crosses were between cultures derived from large ascospores. From each of the two crosses mentioned above, in which all four parents were dissimilar, four or five cultures from large ascospores were obtained. From intercrosses between these cultures, two crosses, in which all four parents were dissimilar, were used to obtain the next generation of cultures.

The essential features of this scheme are that only cultures derived from phenotypically-large ascospores (mean length *c.* 19) are used as parents for the next generation, and that four different strains are used in each set of parental crosses. This mating procedure was used throughout most of the experiment, but on three occasions, the seventh, twelfth and thirteenth generations of selection, progeny from a single cross had to be used to carry on the line.

Crosses	1st generation	Crosses	2nd generation
1×2 ⟶	5 and 6	5×7 ⟶	9 and 10
3×4 ⟶	7 and 8	6×8 ⟶	11 and 12

TEXT-FIG. 1.—Mating procedure in selection experiment for large ascospore size.

The response to selection was at first followed by classifying samples of ascospores from each generation into large (giant) and normal-sized ascospores. The samples of ascospores were taken from at least two, and usually three or four different crosses, in each generation. The total number of ascospores classified was at least 500 in each generation. The result of selection during the first eight generations, based on the frequency counts of large and normal-sized ascospores, has been published previously (Pateman, 1955).

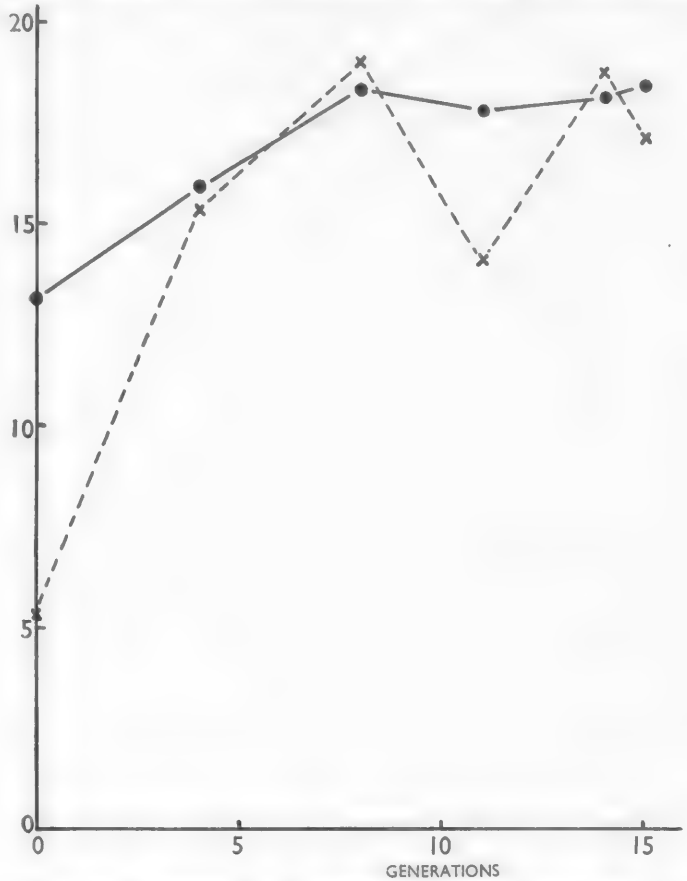
(ii) *Response to selection*

It was realised after the experiment had continued for a number of generations that the response to selection for increased ascospore size was a complex phenomenon. The main factors in this response were as follows : (*a*) A number of asci containing four large ascospores instead of the usual eight normal ascospores were observed. At first the four-spored asci were rare and only one or two of the spores matured in each ascus. But, by the eighth and succeeding generations, there was one ascus containing four large, apparently mature, ascospores per two or three perithecia.

(*b*) During the first generations, the majority of the asci contained eight normal, mature, ascospores. By the eighth and succeeding generations, asci with eight mature ascospores were very rare. After the sixth-eighth generations, only one or two of the ascospores matured

in the great majority of those asci in which eight ascospores were delimited.

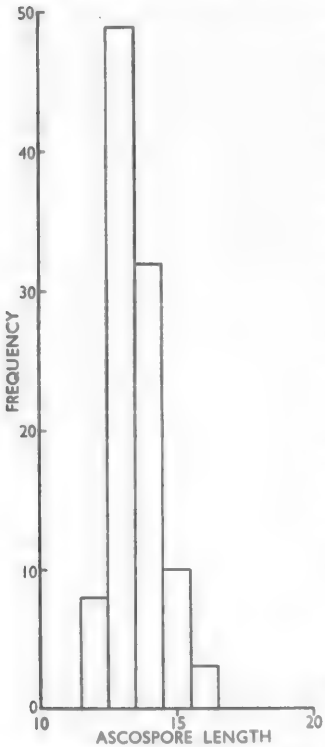
(*c*) There was a general increase in ascospore size which resulted in an increasing number of ascospores intermediate between normal and large, and also in the appearance of rare, very large ascospores.



TEXT-FIG. 2.—The effect of selection for increased ascospore size, together with the corresponding coefficients of variation.

(*d*) As selection proceeded, there was a great increase in the number of asci which aborted at all stages of development, up to that at which ascospores were " cut out " in the ascus but did not mature. In fact, by the eighth generation only about 20 per cent. of the asci contained any mature ascospores at all. The plate illustrates some of the features described in (*a*) to (*d*).

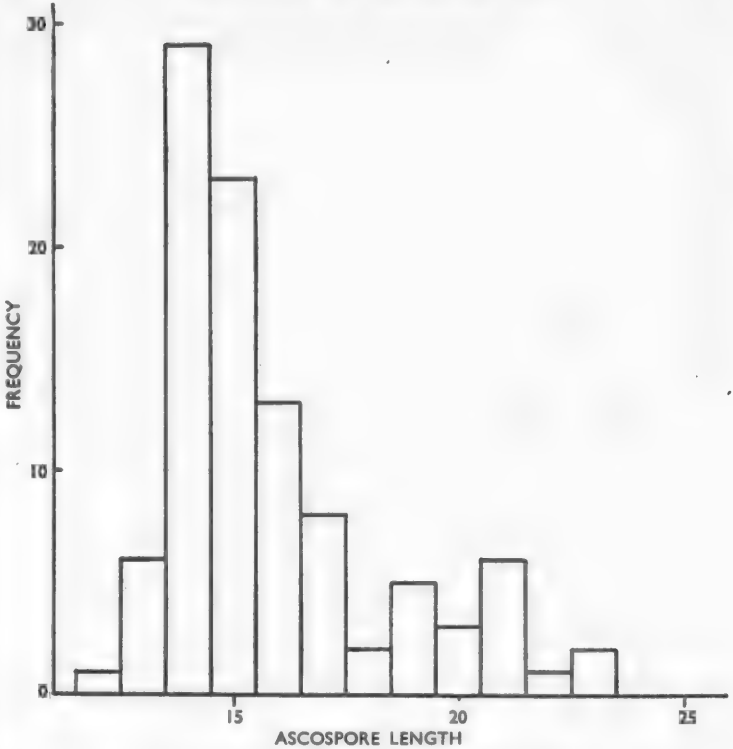
Since selection resulted in the appearance of a new type of ascus in addition to a general increase in size, it was thought desirable to measure individually samples of ascospores and to count the frequencies of four-spored and eight-spored asci. The total number of asci in which half or more of the ascospores were mature was very small. Also, the asci were very fragile and many were broken and rendered unclassifiable by the operation of opening the perithecium. This fragility of the asci varied unpredictably from cross to cross, and the collection of the required data was therefore impracticable. However, samples of 100 ascospores from various generations were measured and some of these results are shown in text-fig. 2. Both the maximum length and breadth of each ascospore were measured, the mean length increased by about 35 per cent., the breadth by about 20 per cent., but only the change in mean ascospore length will be dealt with in this paper. It can be seen from text-fig. 2 that selection resulted in an increase of mean ascospore length from 13-14 to 17·5-18·5. The range of about 1 unit covers the variation usually found between different crosses of the same generation. After the eighth generation there was no further significant change in mean ascospore length. In text-fig. 2, the values of the coefficient of variation ($C =$ (standard deviation of x)$/\bar{x}$, expressed as a percentage) are plotted, together with the corresponding means.



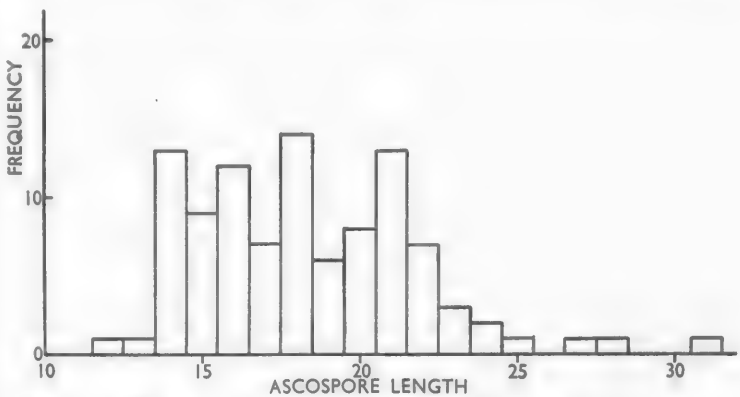
Text-fig. 3.—The frequency histogram of the lengths of a sample of 100 ascospores from a cross between the two stock wild-types, StL. *A* and 1196 *a*.

It can be seen that the coefficient of variation increases from a value of about 5 per cent. for normal crosses to values of 15-18 per cent. after selection for increased ascospore size. This threefold increase in the coefficient of variation, together with the fact that the majority of asci abort at some stage of development and produce few or no viable ascospores, indicates that selection for large ascospores has produced a marked instability of ascus development.

Text-figs. 3-7 show the frequencies of the various size classes in samples of 100 ascospores. It can be seen from text-fig. 3, which is

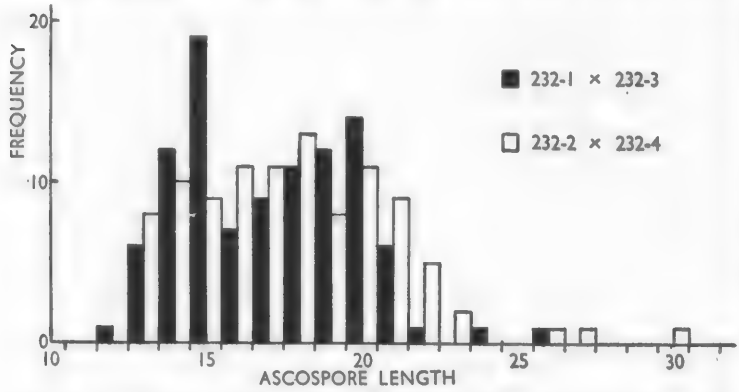


TEXT-FIG. 4.—The frequency histogram of the lengths of a sample of 100 ascospores from a cross between two large-spored strains from the fourth generation of selection.

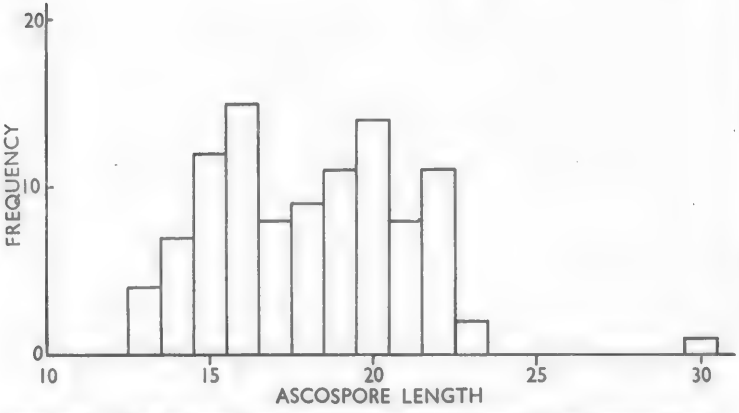


TEXT-FIG. 5.—The frequency histogram of the lengths of a sample of 100 ascospores from a cross between two large-spored strains from the eighth generation of selection.

typical of wild-type crosses, that there is not a great deal of variation about the mean of 13·55, in fact, some 80 per cent. of the ascospores fall into the range 12·5-14·5. Text-fig. 4 is the histogram of the measurements of a sample of ascospores taken from a cross between



Text-fig. 6.—The frequency histograms of the lengths of samples of 100 ascospores from two different crosses between large-spored strains. The four-spored ascus 232 was obtained from a cross between two large-spored strains from the sixteenth generation of selection. The mean ascospore lengths of the two crosses are given in table 5.



Text-fig. 7.—The frequency histogram of the lengths of a sample of 100 ascospores from a cross (234-1 × 234-7) between two normal-spored strains from the sixteenth generation of selection.

two large ascospores obtained after four generations of selection. There is a definite suggestion of bimodality with a peak at 14 and another at about 20. It seems quite clear that the peak at about 20 is due to the proportion of asci which now cut out four large, instead of eight normal ascospores. In addition, there has been an increase in the mean length

of the " normal " ascospores of about 1 unit, with the frequency of the 12·5-13·5 class changing from 49 per cent. in fig. 3 to 6 per cent. in fig. 4. This is a clear indication that selection is having an effect over the whole range of ascospore size and is not just increasing the frequency of asci with four large spores. Text-fig. 5 is the histogram of the measurements of a sample of 100 ascospores, taken from a cross between two large ascospores obtained after eight generations of selection. The obvious bimodality of text-fig. 4 is no longer apparent, all the classes in the range 13·5-21·5 being more or less equally frequent.

TABLE 1

Size of ascospores in two eight-spored and two four-spored asci from crosses between strains obtained after sixteen generations of selection. Asci 233 and 234 were both obtained from the same perithecium.

Ascus	Spore number	Spore size		Ascus	Spore number	Spore size	
		Length	Breadth			Length	Breadth
231	1	15	7	234	1	14	7
	2	16	8		2	15	7
	3	15	8		3	13	7
	4	14	7		4	14	7
	5	15	7		5	...	...*
	6	13	7		6	14	6
	7	14	8		7	16	7
	8	13	7		8	15	6
232	1	20	8	233	1	19	7
	2	19	9		2	22	8
	3	21	9		3	20	9
	4	19	8		4	21	8

* Ascospore did not mature.

The text-figs. 6 and 7 are histograms of the measurements from samples of one hundred ascospores obtained from two different crosses between strains from the sixteenth generation of selection. They show no significant difference from the eighth generation crosses. There was a rapid response for about the first eight generations. Thereafter the selection practised was apparently unable to produce further increase in mean ascospore length.

It is plausible to suggest that there are two populations of ascospores, one derived from four-spored asci the other derived from eight-spored asci, but the size distribution of each now overlaps the other to a considerable extent with the elimination of an intermediate trough in the histogram. This hypothesis is supported by the fact that the great majority of asci which have been observed contained either four or eight ascospores.

In table 1 measurements are given of the ascospores in typical eight-spored and four-spored asci from crosses between strains from the sixteenth generation of selection. It can be seen that there were

at least two ascospores 15 units long and one 16 units long in each of the eight-spored asci : reference to text-fig. 3 shows that there has been an increase in the frequency of rather larger than " normal " ascospores compared with a cross between two unselected strains. Table 2 lists the ascospore measurements of a number of asci which were unusual with respect to the number or range of size of ascospores which they contained. The asci 235 and 236 were obtained from the backcross to the large ascospore parent of a member of the F_1 progeny of a cross between a wild-type and a sixteenth generation large ascospore (see

TABLE 2

Size of ascospores in various unusual types of asci

Ascus	Spore no.	Spore size		Ascus	Spore no.	Spore size		Ascus	Spore no.	Spore size	
		Length	Breadth			Length	Breadth			Length	Breadth
228	1	16	7	237	1	...	...*	235	1	11	6
	2	17	8		2	14	7		2	11	5
	3	17	8		3	15	7		3	12	[illegible]
	4	16	8		4	14	7		4	11	6
	5	17	8		5	15	8		5	12	[illegible]
	6	17	7		6	13	[illegible]		6	12	[illegible]
	7	17	7		7	15	7		7	15	7
	8	16	8		8	19	8		8	16	[illegible]
236	1	16	8	230	1	22	11	229	1	40	9
	2	16	8		2	22	10		...	...	...
	3	15	8		3	21	10		2	23	9
	4	12	7		4	23	11		3	21	9
	5	13	7		...	...	...		...	...	...

* Ascospore did not mature.

later section). All the other asci in table 2 were from crosses between large ascospore strains obtained from the sixteenth generation of selection.

(iii) *Self-fertile strains*

Out of a total of nine large ascospores which germinated and formed the third generation of selected ascospores, two ascospores were observed to grow slowly for about the first two days after germination, then they grew faster and formed healthy vegetative cultures. One of these cultures later developed a few scattered perithecia which produced mature ascospores. In the fourth generation two out of ten large ascospores which germinated were initially slow growing and developed into self-fertile cultures. Another initially slow-growing, self-fertile, large ascospore strain was obtained in each of the fifth and sixth generations of selection. In all, five large ascospores which were initially slow growing and self-fertile and four large ascospores which were initially slow growing, but not self-fertile, were obtained between the third and sixth generations of selection. A total of twenty-five

large ascospores from the self-fertile strains were cultured, fifteen of them were initially slow growing, but none were self-fertile. Nothing further was done with these strains, since it was expected that as selection for larger ascospore size continued, the frequency of self-fertile strains would increase and the nature of these strains could be investigated in more detail later. In fact, no more self-fertile or initially slow-growing strains were obtained from the sixth to the sixteenth generation of selection.

It may be that the phenomenon of slow initial growth immediately after germination was unconnected with the self-fertility of certain strains. But there is a possible explanation which suggests there may have been a connection. It is known to be difficult to obtain heterocaryons in *Neurospora crassa* which carry both mating-type alleles (Garnjobst and Wilson, 1956). Thus if an ascospore was formed which was either heterocaryotic or heterozygous for the mating-type alleles, this might result in uncertain, slow growth immediately after germination. Only in a minority of cases might a strain be able to overcome this initial unbalance and develop normally while still carrying both mating-type alleles and thus become a self-fertile strain. In the majority of instances normal development may occur as the result of the elimination of one or other of the mating-type alleles and a resultant homothallic strain. This would explain why all five self-fertile strains were initially slow growing, but many initially slow-growing strains were not self-fertile.

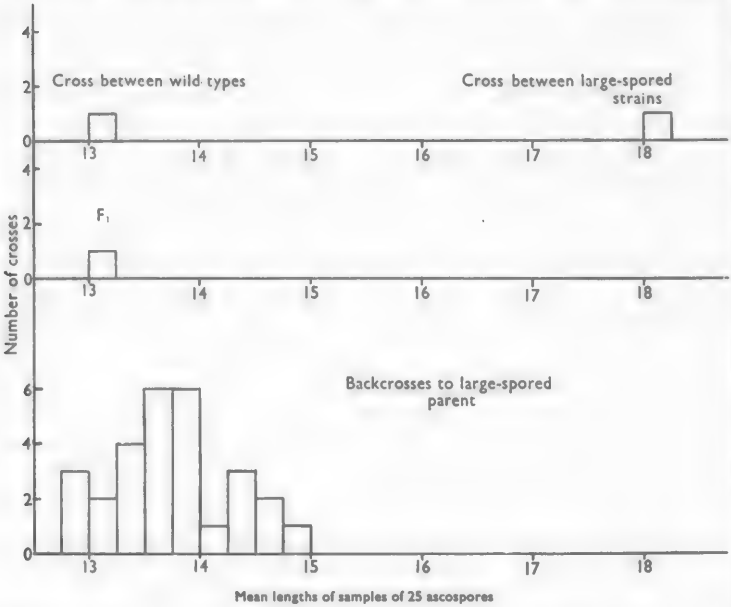
Why the two phenomena, whether connected or not, should have been so transitory is unknown ; it may be that certain rare genes were necessary to produce the effects and at least one of these was eliminated by chance in the process of selection, since the mating population was small.

4. F_1 AND BACKCROSSES FROM LARGE-SPORED STRAINS CROSSED TO WILD-TYPE

The results obtained from crossing large-spored strains from both the third and fifth generations of selection to wild-types and the frequency of large ascospores in the progeny from F_1 crosses have been published previously (Pateman, 1955).

Samples of twenty to 100 ascospores from the F_1 progeny of each of sixty crosses between strains from the fifteenth and sixteenth generations of selection and wild-type have been measured. The mean lengths of the samples of ascospores from these crosses were all within the range 12·8-13·92, the mean length of a sample of 100 ascospores from a typical wild-type cross (see text-fig. 3) was 13·55. It is evident that there is no significant difference between the mean ascospore length of the F_1 and the wild-type crosses. Thus the wild genotype shows complete dominance in crosses with genotypes built up by fifteen or sixteen generations of selection for large ascospore size.

Since the wild genotype showed such complete dominance, it was considered that segregation of nuclear factors affecting type of ascus and ascospore size might be more effectively demonstrated in backcrosses to the large-spored parent than in crosses between different F_1 strains. Accordingly fifty single ascospore strains were isolated from a cross between 233-4 and the StL. *A* wild-type. The ascospore from which 233-4 developed measured 21 ×8 (see table 1). All the F_1 ascospores were of normal size. The fifty F_1 strains were backcrossed



TEXT-FIG. 8.—A comparison of the mean ascospore lengths from wild-type crosses, from crosses between large-spored strains, from an F_1 cross, and from backcrosses to the large-spored parent. The F_1 cross is identical with wild-type crosses. There is a range of intermediate values amongst the twenty-eight different backcrosses.

to 233-4 and a total of twenty-eight fertile backcrosses was obtained. A sample of twenty-five ascospores from each of these backcrosses was measured. The mean length of the ascospores in each of these samples was grouped into class ranges of 0·25 units and text-fig. 8 is a histogram which shows the frequency of the different classes in the total of twenty-eight crosses.

It also shows, for comparison, the mean length of ascospores from crosses of the parental strains to wild-type and to large-spored strains respectively, and the mean length of the F_1 ascospores. The difference between the means of any two samples of ascospores in adjacent classes is statistically significant at about the ·01-·05 level. The

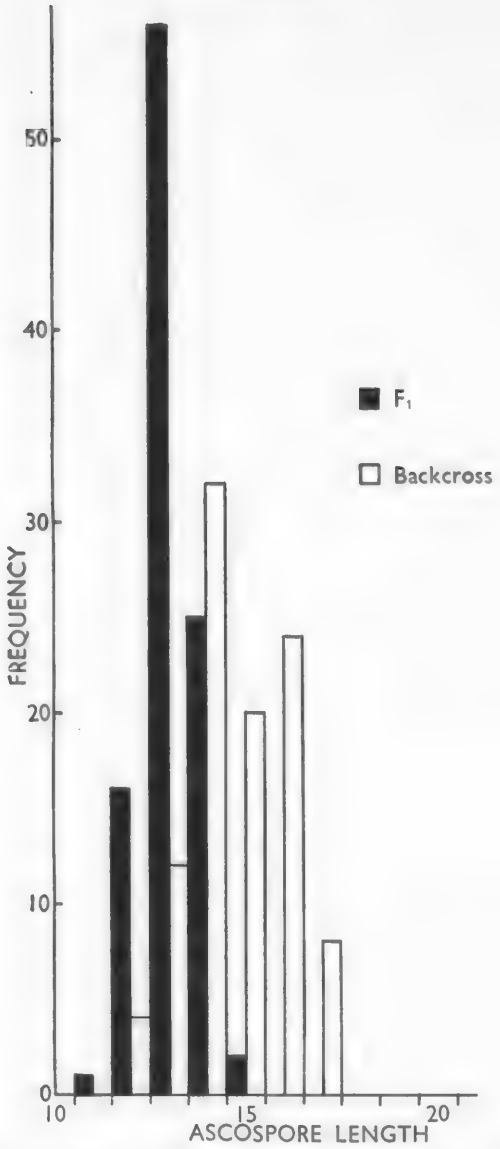
difference between the means of any two samples of ascospores, in different and other than adjacent classes, is significant at less than the ·001 level. It seems reasonable to suggest that segregation of polygenes occurred during the formation of the F_1 ascospores and that this segregation resulted in the wide range of mean ascospore lengths in the twenty-eight backcrosses to the large-spored parent.

In fig. 9 are two histograms. One consists of the measurements of a sample of 100 ascospores with a mean of 13·11 from the cross 233-4 ×StL. *A*. The other consists of the measurements of a sample of 100 ascospores from the backcross of an F_1 strain, 1235, with strain 233-4. This sample has a mean length of 14·72. This illustrates how the genotype of the phenotypically normal, F_1 ascospore, when crossed to that of the large-spored strain 223-4, resulted in a distinctly different distribution of ascospore sizes in the progeny to that found in the F_1 ascospores or in the progeny of wild-type ×wild-type crosses. Also, a four-spored ascus, a five-spored ascus and a typical eight-spored ascus, all from the backcross of an F_1 strain, 1238, to the large-spored parent 223-4 are illustrated in the plate figs. 4-6. This again suggests that segregation of polygenes affecting ascospore size and ascus type occurred during meiosis in the parental cross.

5. APPARENT SEGREGATION OF POLYGENES IN FOUR-SPORED AND EIGHT-SPORED ASCI

One complete eight-spored ascus, numbered 234, and two complete four-spored asci, numbered 232 and 233, were obtained from a cross between two large-spored strains from the fifteenth generation of selection. The sizes of the ascospores in these asci are given in table 1. The four-spored ascus 233 and the eight-spored ascus 234 were both obtained from the same perithecium. The four possible crosses between members of the same ascus 232 and the four possible crosses between members of the ascus 233 were set up. Also, all the possible crosses between the member strains of ascus 234 were set up. Samples of 100 ascospores from each of these crosses were measured. There were apparently significant differences in the mean length of ascospores from various crosses between members of the same ascus. It was considered that these differences might be the result of segregation of polygenes during meiosis.

In order to confirm this apparent segregation of polygenes during the formation of the asci, the following experiments were carried out. The four possible crosses between the four strains of ascus 232 were set up with five replicates of each type of cross. All the crosses were set up at the same time, on crossing slopes made from the same batch of medium and were kept close together in the culture room. These precautions were taken in order to minimise the between-replicate variation due to environmental differences. A sample of twenty ascospores from each of the twenty crosses was measured, the mean



TEXT-FIG. 9.—The frequency histograms of samples of 100 ascospores from an F_1 and from a backcross to the large-spored parent.

length of each of these samples is given in table 3. The analysis of variance of these results is given in table 4. The mean square (MS = 9·593) for the comparison between the four different types of cross is less than the mean square for comparison of replicate crosses (MS = 17·255). The sum of squares for comparisons between parental strains has been partitioned into three components, each with one

TABLE 3

Mean lengths of samples of ascospores from crosses between strains from ascus 232

Cross	Mean length of samples of twenty ascospores	Joint mean of samples
232-1 × 232-3	17·40 16·85 17·30 17·50 16·25	17·06
232-1 × 232-4	18·40 16·55 17·15 18·50 17·45	17·61
232-2 × 232-3	16·95 17·10 17·65 18·00 16·95	17·33
232-2 × 232-4	16·50 16·45 17·65 18·35 19·85	17·76

degree of freedom, but none of these gives a significant variance ratio. That is, there is no difference between any of the twenty crosses which can be attributed to the parental strains. Although precautions were taken to minimise variation due to environmental causes, any difference between crosses which may have been due to different parental genotypes was small by comparison with the variation between replicate crosses.

A similar experiment was made with strains from the eight-spored ascus 234 and in this experiment, also, the differences between crosses with different parents were no greater than the differences between replicate crosses. In view of these experiments with sets of replicated crosses, it seems that the apparently significant differences previously described may have been due to the greatly increased variance in crosses between strains from the sixteenth generation of selection.

This does not of course apply to the data from the backcrosses, which provide unequivocal evidence of segregation of polygenes.

6. RECIPROCAL CROSSES

A number of reciprocal crosses between strains derived from large ascospores from the fifteenth and sixteenth generations of selection, and stock wild-types were set up. It was observed that there were small but significant differences in the mean ascospore lengths of samples of ascospores from reciprocal crosses. In every case the mean ascospore length was greater when the wild-type strain was used as

TABLE 4

Analysis of variance of ascospore length in crosses between the four strains from ascus 232

Item	*n*	Mean square	F	Probability
1 *v.* 2	1	4·40*	...	...
3 *v.* 4	1	24·01	2·6648	>0·1
Interaction	1	0·37*	...	...
Total between strains	3	9·593	1·0647	>0·2
Between replicates	16	17·255	1·9150	<0·05
Total between crosses	19	16·045	1·7807	<0·05
Error	380	9·010		
Total	399			

* Not significantly smaller than error mean square.

the maternal parent, than in the reciprocal cross. The mean ascospore lengths of samples of 100 ascospores from six different sets of reciprocal crosses are given in table 5, these include the four sets of reciprocal crosses made between the strains from the four large ascospores of ascus 233 and stock wild-types.

In addition, four different sets of reciprocal crosses between wild-type strains and strains derived from four of the normal-sized ascospores derived from ascus 234 are given in table 5. It can be seen from table 5 that there is no indication of any difference in the reciprocal crosses when strains derived from ascospores of normal size are crossed with wild-types. Yet ascus 234 was obtained from a cross between two large ascospores from the fifteenth generation of selection. In fact, ascus 233, which contained four large ascospores, and ascus 234 which contained eight ascospores of normal size (see table 1) were both from the same perithecium. There was a difference between reciprocal crosses only when a strain derived from a phenotypically-large ascospore was crossed with a wild-type strain. An analysis of variance

of the results obtained from the six sets of reciprocal crosses of large-spored strains given in table 5 shows that the reciprocal difference is significant ($F = 45{\cdot}25$, P *c.* 001).

A further experiment was made with five replicates of each of the reciprocal crosses between the four strains from ascus 233 and the wild types StL. *A* and 1196*a*. The total of forty crosses was set up in the most uniform fashion possible, in order to minimise environmental variation. A sample of twenty ascospores from each cross was measured and the results are given in table 6. The analysis of variance of the results is given in table 7.

It can be seen from table 7 that the difference between reciprocal crosses is significant. The mean square for the individual or residual

TABLE 5

Size of ascospores obtained from reciprocal crosses

Cross	Mean length of samples of 100 ascospores	Cross	Mean length of samples of 100 ascospores
1077 × StL.	12·95	StL. × 1077	13·45
1078 × StL.	13·00	StL. × 1078	13·39
233-1 × 1196	13·22	1196 × 233-1	13·59
233-2 × 1196	13·18	1196 × 233-2	13·92
233-3 × StL.	13·56	StL. × 233-3	13·83
233-4 × StL.	13·11	StL. × 233-4	13·51
234-1 × StL.	13·21	StL. × 234-1	13·43
234-2 × StL.	13·18	StL. × 234-2	13·01
234-3 × StL.	13·38	StL. × 234-3	13·29
234-4 × StL.	13·30	StL. × 234-4	13·40

Strains 1077, 1078, 233-1, 233-2, 233-3 and 233-4 are derived from large ascospores.
Strains 1196 and StL. are stock wild types.
Strains 234-1, 234-2, 234-3 and 234-4 are derived from normal-sized ascospores obtained from a cross between two strains from the fifteenth generation of selection.

variation is used as the denominator in the computation of F for the reciprocal effect, since it is larger than the mean square for the variation between replicate crosses.

The parental-genotype × reciprocal-difference interaction was also significant. The seven degrees of freedom for the eight sets of five replicate crosses may be partitioned individually. One of the three degrees of freedom for differences between parental genotypes may be used for the strain 233-1 *versus* strains 232-2, 233-3 and 233-4 comparison. The interaction of this comparison with the reciprocal effect gives a sum of squares of 5·7 out of the total of 7·18 for the whole parental-genotypes × reciprocal-difference interaction. The value of F for this degree of freedom is 9·9, P *c.* 001. In other words, there is a significant interaction between strain 233-1 and the reciprocal difference, but no significant interaction between any of the other

three strains from ascus 233 and the reciprocal effect. The reason for this is unknown.

It was necessary to use two wild-type strains of different mating types in the experiment. Strain 1196*a* was derived from seven generations of backcrossing to StL. *A*, so the two strains should have been largely isogenic. In fact, the sum of squares for the comparison between the two wild-type parents was 0·1, MS = 0·1, F = 5·85, P > 0·2.

TABLE 6

Mean length of ascospores from replicate reciprocal crosses

Cross	Mean length of samples of 20 ascospores	Joint mean of samples	Cross	Mean length of samples of 20 ascospores	Joint mean of samples
233-1 × 1196	12·95 13·00 12·80 13·00 13·15	12·98	1196 × 233-1	13·60 13·60 13·65 13·60 13·80	13·65
233-2 × 1196	12·80 13·00 13·10 13·30 13·05	13·05	1196 × 233-2	13·25 13·45 13·35 13·65 13·50	13·44
233-3 × StL.	13·30 13·35 13·10 13·15 13·35	13·25	StL. × 233-3	13·45 13·25 13·70 13·35 13·25	13·40
233-4 × StL.	13·15 13·15 13·25 13·10 13·00	13·13	StL. × 233-4	13·50 13·30 13·50 13·40 13·45	13·43

That is, there was no significant effect which could be attributed to the wild-type parental strains.

7. DISCUSSION

The increased range of ascospore size found in the backcross progeny compared with the F_1 indicates that a considerable number of genes differentiate the wild-types and the strains obtained after fifteen to sixteen generations of selection for increased ascospore size. This fact, together with the steady response to selection, suggests that a system of polygenes, which controlled to a considerable extent the number and size of ascospores in the asci, had been built up as a result of the selection.

Although it was not possible to estimate them independently, it is clear that there were three main results of selection : an increase

in the average size of all ascospores, the development of asci containing four large ascospores instead of eight normal-sized ascospores, and developmental instability reflected in an increasing proportion of aborted ascospores and asci. The three main effects cannot really be considered separately, since they are obviously components of an integral phenomenon, yet they are suggestive of three main levels at which selection may have been effective. It is plausible to suggest

TABLE 7

Analysis of variance of ascospore length in replicate reciprocal crosses between the four members of ascus 233

Item	n	Mean square	F	Probability
Second order interactions :				
R.E. × (1 *v.* 2+3+4) . .	1	5·70	9·9 7·7*	<0·01 0·1-0·2
R.E. × (other comparisons) . .	2	0·74	1·26	>0·2
Total interaction . . .	3	2·39	4·061	<0·01
Between large-spored parent strains	3	0·26†	...	...
Reciprocal effect (R.E.) . .	1	28·50	48·428 11·92‡	<0·001 <0·05
Total between eight different types of cross	7	5·21	8·853	<0·001
Between replicate crosses . .	32	0·3568§	...	...
Total between all crosses . .	39	1·228	2·086	<0·001
Error	760	0·5885		
Total	799			

* This variance ratio was calculated using the mean square (0·74) for the rest of the second order interaction as the denominator.

† Not significantly smaller than the error mean square.

‡ This variance ratio was calculated using the total interaction mean square as denominator.

§ Significantly smaller than the error mean square at the ·05 level.

that the initial source of genetic variability in the fungus was a mutation, a rare recombination, or both, which primarily affected the developmental stability of ascospore and ascus development. Consequently, environmental variation, which would have no appreciable effect on the development of the normal wild-type, might occasionally be sufficient to push development over some threshold in cell metabolism which determined whether or not mitosis should follow meoisis in the ascus initial. In this way, in a small proportion of asci, spore walls might be delimited around the four meiotic, nuclear products, instead of the usual eight ascospore initials. The fact that the ascospores in

this type of ascus were large, might be simply a result of there being more cytoplasm available for inclusion in four instead of eight ascospores. It is probably significant that in the initial generations of selection no asci with four mature, large, ascospores were observed, but only single large ascospores together with aborted ascospore initials in each four-spored ascus. It suggests that the genotypic control of the development of the four-spored type of ascus was not at first sufficiently stable to produce more than an occasional single, large mature ascospore.

As selection for phenotypically-large ascospores proceeded, three at least partially independent systems of modifying genes might be built up, if the genetic variance was available. One system would be responsible for an overall increase of ascospore size as such. Another might lower still further the threshold at which the switch from the eight-spored to the four-spored type occurs, with a resultant increase in the number of the four-spored type and in the number of aborted asci. A situation analogous to this has been discussed by Waddington (1953). The third system would be responsible for an increased stability of development in some of the four-spored asci with an increase in the number of mature, large ascospores. It has been demonstrated by Mather (1953) that developmental stability in *Drosophila* is under genetic control and can be affected by selection. All of these systems would contribute in some measure to the increase in frequency of phenotypically-large ascospores.

This explanation of the mode of operation of selection is, of course, only speculative. But there is one aspect of the response to selection which seems fairly clear. The " plateau " at about 18-19 units, when after the previous rapid response the method of selection practised apparently became ineffective, was probably a result of the developmental instability which itself formed part of the response to selection. At this level the variation in ascospore size due to slight and uncontrollable environmental causes, was a good deal larger than that due to genotypic differences. Consequently the ascospore phenotype no longer provided a sufficient indication of the ascospore genotype for the method of selection to be effective in promoting a further increase of size. This kind of situation in *Drosophila* has been discussed by Thoday (1955).

The difference between reciprocal crosses of cultures from phenotypically-large ascospores and wild-type strains, is interesting for two reasons. First, because it is the cross with the wild-type as the maternal parent which gives the greater mean ascospore length in the progeny and not the other way about. Second, there was no reciprocal effect in crosses with wild-types of cultures from phenotypically normal-sized ascospores, such as those of ascus 234, which are derived from large-spored strains produced by fifteen to sixteen generations of selection. Yet, crosses between cultures from these phenotypically normal-sized ascospores, see table 4, show that their genotypes are

very similar to those of the phenotypically-large ascospores. Present information does not suggest any obvious explanation of this reciprocal effect. It is obvious that the difference between reciprocal crosses is closely connected with the control of ascus development. It remains to be discovered at which stage in development, and in what way, the number and size of the ascospores to be delimited in the ascus is determined. If this were known it would probably provide an explanation of the reciprocal difference. Alternatively, it may be that further experimental investigation of the reciprocal effect will increase our understanding of ascus development.

In addition to three species with eight-spored asci like *Neurospora crassa*, the genus *Neurospora* contains three species, such as *Neurospora tetrasperma*, which have four-spored asci. It is usually considered that the four-spored species of *Neurospora* are derived from the eight-spored forms (Dodge *et al.*, 1950). When postulating the evolution of a species with four large ascospores per ascus from one with eight smaller ascospores per ascus, it is not easy to suggest any selective advantage which might result in such an evolution. But the progress of the selection experiment can be used to suggest one way in which the four-spored forms may have evolved from one or more of the eight-spored species.

It may be that the initial stage of divergence from an ancestral eight-spored form was not directly connected with ascospore size or number of ascospores per ascus at all. If some change in the environment occurred which exceeded the range of conditions to which the ancestral eight-spored species was well adapted, this might confer a selective advantage upon a genetic variant which possessed greater adaptability in some way at the biochemical level in cell development. The altered physiological conditions in the cells of the better adapted genetic variant growing in the different environment might indirectly result in a switch from the eight-spored to the four-spored type of ascus development. If such a switch occurred in a significant proportion of asci it might well have the following consequences. At first a large number of asci would probably abort at various stages, since the new type of ascus is not likely to have been initially as stable in development as the ancestral eight-spored type of ascus perfected by a long period of natural selection. This ascus abortion, together with reduction of the number of ascospores per ascus by a half without a corresponding doubling of the number of asci would cause a marked decrease in the number of viable ascospores. This reduction would confer a selective advantage on any modifying genes which increased the proportion of viable ascospores in the four-spored type of ascus. The production of homothallic ascospores might also have had a selective advantage in a species where the number of ascospores was limiting. Thus a species regularly producing four self-fertile ascospores per ascus may have come into being. This explanation of how a four-spored species may have evolved is obviously derived in many

respects from the way in which the selection experiment is known to have proceeded. There are also several further points which ought to be mentioned in this connection.

The gene E, reported by Dodge *et al.* (1950) in *Neurospora tetrasperma*, affects some vital step in the metabolism of the ascus initial, since it behaves as a dominant lethal resulting in the abortion of practically all the asci when a strain carrying it is mated on the usual media. But on a special potato steep medium the asci of this type of cross do not abort, instead they delimit eight small ascospores. This mutant strain illustrates the fact that some biochemical step in the cell can determine the type of ascus development, and that this metabolic step is at least partially controlled by the gene E. It will be remembered that the hypothesis outlined above suggests that an adaptive physiological change, made possible by genotypic variation, was the prime cause of evolutionary divergence, while the morphological changes were an indirect result.

The work of Dodge (1928) shows that the inheritance of the four-spored and eight-spored types of ascus in crosses and backcrosses between *Neurospora sitophila* and *Neurospora tetrasperma* is at least partly polygenic. The existence of these polygenic differences between an eight-spored and a four-spored species increases the plausibility of the suggestion that the build-up of some such system of modifying genes comprised part of the second stage of the evolution of the four-spored species.

It is improbable that the origin of such four-spored species as *Neurospora tetrasperma* will ever be known with certainty. But at least a hypothesis can be proposed which is in accord with what is at present known, from three independent pieces of experimental work, about the relevant genetic nature of *Neurospora* species.

8. SUMMARY

(1) Selection for increased ascospore size in *Neurospora crassa* for sixteen generations had the following main results :

(*a*) The mean ascospore size increased from about $27\mu \times 15\mu$ to about $37\mu \times 18\mu$.

(*b*) The eight-spored type of ascus characteristic of wild-type *Neurospora crassa* became very rare, and asci containing four large ascospores were produced by crosses between the selected strains. Strains from the sixteenth generation of selection produced about one ascus with four large, apparently mature ascospores in every three perithecia.

(*c*) There was a great increase in the frequency of aborted asci and ascospores. In crosses between strains from the sixteenth generation of selection, only about 20 per cent. of the asci contained mature ascospores.

(*d*) The method of selection practised became ineffective after about eight generations, and there was no significant

Plate

The effects of selection for large ascospore size in *Neurospora crassa*.

FIG. 1.—The F_1 asci from a cross between the large-spored strain 233-2 and a wild-type 1196. All the asci contain eight normal-sized spores and are indistinguishable from those produced by a wild-type × wild-type cross. ×45.

FIG. 2.—Part of the contents of a perithecium from a cross between the two large-spored strains 233-2 and 233-4: both strains came from the sixteenth generation of selection. There are several of the four-spored type of ascus, each containing one, two or three mature large spores. There is one ascus which contains eight normal-sized spores, but none of these are mature. ×50.

FIG. 3.—Part of the contents of a perithecium from a cross between the two normal-spored strains 234-3 and 234-7, both strains came from the sixteenth generation of selection. This shows the close similarity to the type of cross illustrated in fig. 2. ×50.

FIG. 4.—The four-spored ascus is from the backcross of an F_1 strain, 1238, to the large-spored parental strain, 233-4. The sizes of the spores are 21×8, 23×8, 20×8 and 20×8. ×280.

FIG. 5.—The five-spored ascus is from the same backcross as in fig. 4. The sizes of the spores are 16×8, 16×8, 15×7, 12×7 and 13×7. ×300.

FIG. 6.—The eight-spored ascus is from the same backcross as figs. 4 and 5. It is typical of the majority of complete asci in the backcross. The sizes of the spores are 14×7, 14×7, 13×7, 12×7, 14×7, 14×7 and 13×6. ×225.

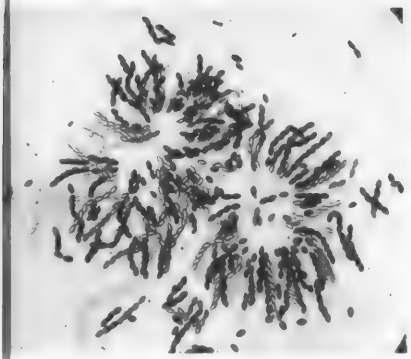


FIG. 1

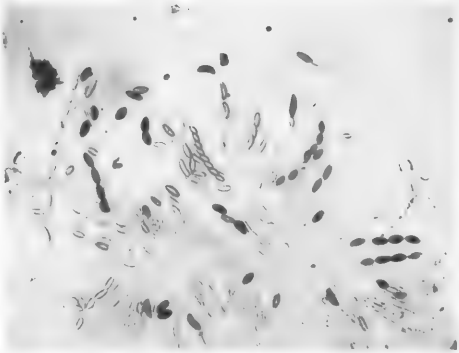


FIG. 2

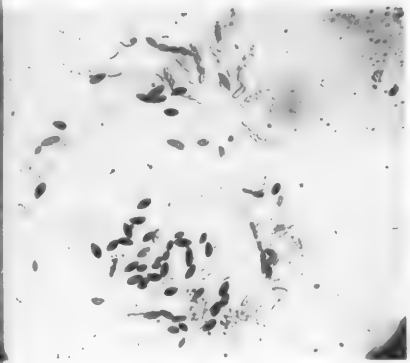


FIG. 3

FIG. 4

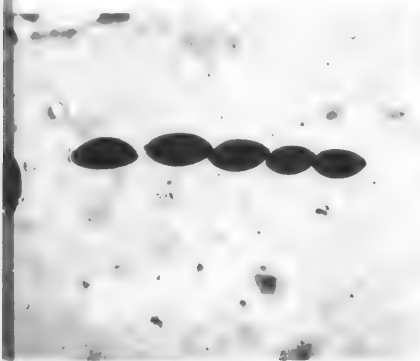


FIG. 5

FIG. 6

deviation from the level reached in the eighth generation during the succeeding ninth to sixteenth generations of selection.

(2) In crosses between wild-type strains and large-spored strains obtained after selection, the F_1 asci all contained eight normal ascospores and were indistinguishable from those produced by wild-type crosses. There was no indication of unusual ascus or ascospore abortion in F_1 progeny. Thus the wild-type genotype showed complete dominance.

(3) It is considered that the inheritance of ascospore size and type of ascus development in *Neurospora crassa* is chiefly controlled by a system of polygenes for the following three reasons. The mean ascospore size steadily increased for eight generations in response to selection. There was considerable variation in individual ascospore size in the progeny from crosses between large-spored strains. There was a wide range of mean ascospore sizes in twenty-eight different backcrosses to a large-spored parent strain.

(4) There was a significant difference between reciprocal crosses of large ascospore strains from the sixteenth generation of selection with wild-type strains. When the wild-type strain was the maternal parent the mean length of the F_1 ascospores was about 1 μ greater than the mean length of the F_1 ascospores from the reciprocal cross.

A hypothesis is proposed to account for the results observed in the selection experiment. An explanation is also suggested of one way in which selection may have resulted in the evolution of the four-spored species of *Neurospora* from one or more ancestral eight-spored species.

Acknowledgment.—I am indebted in this work to the Agricultural Research Council for some technical assistance, which was made possible by a grant made by them to Dr J. M. Thoday.

9. REFERENCES

BEADLE, G. W., AND TATUM, E. L. 1945. Methods of producing and detecting mutations concerned with nutritional requirements. *Amer. J. Bot.*, *32*, 678-686.

DODGE, B. O. 1928. Production of fertile hybrids in the ascomycete *Neurospora*. J. Agric. Research, 36, 1-14.

DODGE, B. O., SINGLETON, J. R., AND ROLNICK, A. 1950. Studies on lethal E gene in *Neurospora tetrasperma*, including chromosome counts also in races of *Neurospora sitophila*. *Proc. Amer. Phil. Soc.*, *94*, 1. 38-52.

GARNJOBST, L., AND WILSON, J. F. 1956. Heterocaryosis and protoplasmic incompatibility in *Neurospora crassa*. *Proc. nat. Acad. Sci.*, Wash., *42*, 613-618.

MATHER, K. 1953. Genetical control of stability in development. *Heredity*, *7*, 297-336.

PATEMAN, J. A. 1955. Polygenic inheritance in *Neurospora*. *Nature*, Lond., *176*, 1274-1275.

THODAY, J. M. 1955. Balance, heterozygosity and developmental stability. *Cold Spr. Harb. Symp. quant. Biol.*, *20*, 318-326.

WADDINGTON, C. H. 1953. Genetic assimilation of an acquired character. *Evolution*, *7*, 118-126.

WESTERGAARD, M., AND MITCHELL, H. K. 1947. *Neurospora* V. A synthetic medium favouring sexual reproduction. *Amer. J. Bot.*, *34*, 573-577.

CROSSING-OVER IN MICE HETEROZYGOUS FOR *t*-ALLELES

MARY F. LYON and RITA J. S. PHILLIPS
Medical Research Council Radiobiological Research Unit, Harwell, Didcot, Berks

Received 21.iii.58

1. INTRODUCTION

For studies of mutagenesis in the house mouse it would be very useful if there were chromosome changes acting as crossover suppressors, which could be used to build up stocks resembling in function the C*l*B and Muller-5 stocks of *Drosophila melanogaster*. The only crossover suppressors so far known are some of the recessive lethal *t*-alleles in linkage group IX. The experiments reported in this paper were

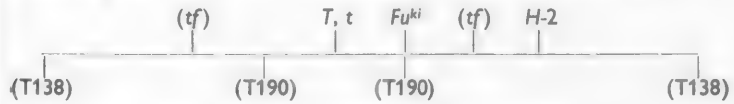


Fig.—Known loci in linkage group IX of the mouse.

T	Brachyury. Dominant. Heterozygote has short tail, homozygote dies *in utero*.
t^0, t^1, ..., t^n	Recessive alleles at the *T*-locus. *Tt* heterozygotes tailless, +*t* normal. Homozygotes for some alleles lethal, some viable and normal tailed. Some alleles show non-Mendelian segregation in males, some suppress crossing-over.
Fu^{ki}	Kinky. Dominant. Heterozygote has kinky tail, homozygote dies.
H-2	Histocompatibility. Determines the antigenic specificity of tissues (*e.g.* tumours) as detected by transplantation studies.
tf	Tufted. Recessive. Affects hair growth.
T138	Chromosome translocation involving linkage groups II and IX. The break in group IX is remote from *T*.
T190	Chromosome translocation involving linkage groups IX and XIII. The break in group IX is near to *T* and the translocated chromosome carries t^6.

The position of *tf*, T138 and T190 relative to *Fu* and *H*-2 had not been found at the start of this work.

intended to extend knowledge of the crossover-suppressing effect of *t*-alleles in the hope of using them in stocks for studies of mutagenesis. The fig. gives a diagram of the loci in group IX as known at the start of these experiments and describes the genes and translocations concerned.

Dunn and Caspari (1945) and Dunn and Gluecksohn-Waelsch (1953*b*) showed that some *t*-alleles suppress crossing-over between *T* and the nearby locus *Fu*. Until recently *T*, *Fu* and *H*-2 were the only known loci in linkage group IX. This made more detailed studies of the crossover-suppressing effect of *t*-alleles technically difficult since *T* and *Fu* both affect the same organ, the tail, and the segregation of *H*-2 alleles can only be observed by a laborious technique of tumour transplantation. Recently, however, tufted, *tf*, was found to lie in this linkage group (Lyon, 1956). In addition translocation

T138, described by Carter, Lyon and Phillips (1955, 1956), involves linkage groups II and IX, so that in mice carrying this translocation *T* shows linkage with dilute, *d*, of group II.

The *t*-alleles used in the present experiments were t^6 and t^{13}. t^6 was found by Carter and Phillips (1950) and reported by Dunn and Gluecksohn-Schoenheimer (1950) not to differ from the previously known lethal allele, t^0, which is a crossover suppressor. The allele t^{13} was found by Dunn and Gluecksohn-Waelsch (1953*a*) in a normal-tailed exception in a balanced lethal line $Tt^1 \times Ttt^1$. It is viable when homozygous and its effect on crossing-over had not been tested at the start of this work. Crossing-over has been studied between the *T*-locus and *tf* in t^6 and t^{13} heterozygotes and among *T*, t^6, *tf* and *d* in **T138** heterozygotes. In addition, a note is made of a possible simultaneous mutation and crossover in translocation **T190** which has a bearing on the observed high mutation rate of *t*-alleles (Dunn, 1956).

2. CROSSES AND RESULTS

(i) *Crossing-over in heterozygotes for* tf *and* t^6

A $T+$ *tf/tf* female was crossed with a $+t^6$ $++^{tf}$ male and Tt^6 $+tf$ progeny were backcrossed to $++^T$ *tf/tf* animals. Offspring from these matings were used to make crosses of other types. The data are shown in table 1. From Tt^6 $+tf$ heterozygotes there were no crossovers, while from $+t^6$ $+tf$ heterozygotes two crossovers were obtained, one from a female and one from a male heterozygote. In this cross the presence or absence of the *t*-allele can only be scored in those young which inherited *T* from their other parent. Hence, only young carrying *T* give linkage information and the recombination in $+t^6$ $+tf$ heterozygotes is 2/162 or 1·23 per cent. with upper and lower fiducial limits at the 5 per cent. significance level of 0·15 and 4·46 per cent. The data from males and females have been combined as no significant sex difference in recombination is indicated. Combining the data from male and female $+t^6/tfT$ heterozygotes, the upper fiducial limit of recombination at the 5 per cent. significance level is 1·01 per cent. Since the recombination observed in $T+$ $+tf$ heterozygotes was $8{\cdot}3 \pm 1{\cdot}5$ per cent. (Lyon, 1956) t^6 clearly suppresses crossing-over in the $T-tf$ chromosome region.

(ii) *Crossing-over in heterozygotes for* tf *and* t^{13}

Three Tt^{13} $++^{tf}$ males, kindly given by Professor L. C. Dunn, were crossed with $T+$ *tf/tf* females and Tt^{13} $+tf$ progeny were backcrossed to $++^T$ *tf/tf* animals. The results are shown in table 1. The observed recombination is $11{\cdot}0 \pm 2{\cdot}00$ per cent. in females and $2{\cdot}92 \pm 1{\cdot}44$ per cent. in males. A test for heterogeneity of the proportions of recombinants and non-recombinants in the two sexes gives $\chi^2 = 7{\cdot}62$, $P < 0{\cdot}01$. No such sex difference in recombination was observed in $T+$ $+tf$ heterozygotes. For comparison, the data given in the earlier

paper on tufted, together with some additional data are included in table 1. In this case male heterozygotes show slightly higher recombination, but the difference does not approach statistical significance. Hence, t^{13} clearly has some effect on crossing-over in the $T-tf$ region, but it cannot be considered as a crossover suppressor.

TABLE 1

Recombination in the T−tf *region in* t^6 *and* t^{13} *heterozygotes*

Heterozygous parent		Phenotypes of offspring								Recombination per cent.	Upper fiducial limit per cent.	Lower fiducial limit per cent.
Type and mating	Sex											
		$+_6T$	$+t^6$	tfT	tft^6				Total			
$+t^6/tfT\times$ $tf+/tf+$	♀	...	44	48	...				92	0·0	4·01	...
	♂	...	158	114	...				272	0·0	1·36	...
	♀ and ♂	...	202	162	...				364	0·0	1·01	...
		$+T+$	$+Tt^6$	$tfT+$	$tfTt^6$	...	$++$	$tf+$	Total			
$+t^6\ tf+\times$ $tfT/tf+$	♀	1	38	72	...	76	75		262	0·90	...	...
	♂	1	29	21	...	46	19		116	1·96	...	...
	♀ and ♂	2	67	93	...	122	94		378	1·23	4·46	0·15
										Recombination per cent.	S.E.	
		$+T$	$+t^{13}$	tfT	tft^{13}				Total			
$+t^{13}\ tfT\times$ $tf+/tf+$	♀	11	105	114	16				246	11·00	2·00	...
	♂	3	39	94	1				137	2·92	1·44	...
										Recombination per cent.	S.E.	
		$+T$	$++$	tfT	$tf+$				Total			
$T\ tf+\times$ $tf+/tf+$	♀	117	8	14	122				261	8·43	1·72	...
	♂	70	4	9	58				141	9·22	2·44	...

(*iii*) *The effect of* t^6 *on crossing-over in* **T138** *heterozygotes*

In translocation **T138** the chromosome break in linkage group II is about 20 centimorgans from dilute, *d*, and in group IX the position of the break is such that *T* and *d* show 40 per cent. recombination in translocation heterozygotes (Carter, Lyon and Phillips, 1955, 1956). Tests were made to find whether t^6 suppresses crossing-over between *T* and the translocation break, by linkage tests between *T* and *d* in the presence and absence of t^6. The tests were designed to run concurrently, and to yield similar phenotypic classes, to minimise viability disturbances in the linkage data. The types of heterozygotes used and results obtained are shown in table 2.

In female heterozygotes carrying t^6 there is a clear reduction in crossing-over between *T* and *d*, from $42{\cdot}5\pm7{\cdot}8$ per cent. in the absence

of t^6 to $22{\cdot}2\pm3{\cdot}4$ per cent. in its presence. In male heterozygotes there is again a reduction, but a smaller one, from $40{\cdot}7\pm3{\cdot}9$ per cent. to $32{\cdot}4\pm3{\cdot}9$ per cent. To find whether the suppression of crossing-over between the *T* locus and the translocation break was complete, female progeny of these crosses were mated to normal males and tested for semi-sterility by counts of live and dead embryos to detect the presence of the translocation. (The criteria used for diagnosing semi-sterility were those of Carter, Lyon and Phillips, 1955.) Knowing the genotype of the tested animals with respect to *T* and *d*, the *T*—**T138** and *d*—**T138** recombinations could then be found. This method of estimating linkage by finding the fertility of female progeny gave only an approximate estimate of the recombination since there are two sources of error in testing females for semi-sterility. First, Carter, Lyon and Phillips estimated that 1 per cent. of wrong diagnoses would be made if only one uterus were examined using their criteria, and second, an appreciable proportion of females cannot be diagnosed either as semi-sterile or as fully fertile since they have an intermediate proportion of dead embryos and come under the category of "test inconclusive". It is likely that these inconclusive animals will not include equal proportions of actual semi-steriles and fully fertiles and this may lead to disturbance of the observed ratios. Table 3 shows that in the presence of t^6, crossing-over between *T* and **T138** still occurs, indicating that the crossover-suppressing effect of t^6 does not extend throughout the chromosome. The *d*—**T138** recombination shows no significant difference in *T*+ and *Tt*^{6} heterozygotes ; so there is no evidence that

TABLE 2

Recombination in **T138** *heterozygotes in the presence or absence of* t^6. *All matings were backcrosses to* **T138** **T138**+ +^{T}dd *animals*

Heterozygous parent		Phenotypes of offspring					Recombination per cent.	S.E.
Type	Sex	*T*+	*Td*	++	+*d*	Total		
(1) **T138** *T*+/++*d*	♀	11	6	11	12	40	42·5	7·8
	♂	16	4	7	8	35	...	...
(2) +*T*+/**T138**+*d*	♀	38	24	31	24	127	...	...
(1)+(2)	♀♂	54	28	38	42	162	40·7	3·9
(3) **T138** *t*^{6}*d*/+*T*+	♀	32	4	2	16	54	...	...
(4) +*t*^{6}*d*/**T138** *T*+	♀	7	2	16	29	54	33·3	6·4
(3)+(4)	♀	26	11	10	26	73	...	...
(5) **T138** *Td*/+*t*^{6}+	♀	58	15	12	42	127	21·3	3·6
	♂	4	9	10	3	26	26·9	8·7
	♂	4	5	57	25	91	31·9	4·9
				OC	RC			
Total (3)—(5)	♀			119	34	153	22·2	3·4
	♂			98	47	145	32·4	3·9

OC = old combinations ; RC = recombinations.

and *d*—**T138** recombinations could then be found. This method of estimating linkage by finding the fertility of female progeny gave only an approximate estimate of the recombination since there are two sources of error in testing females for semi-sterility. First, Carter, Lyon and Phillips estimated that 1 per cent. of wrong diagnoses would be made if only one uterus were examined using their criteria, and second, an appreciable proportion of females cannot be diagnosed either as semi-sterile or as fully fertile since they have an intermediate proportion of dead embryos and come under the category of "test inconclusive". It is likely that these inconclusive animals will not include equal proportions of actual semi-steriles and fully fertiles and this may lead to disturbance of the observed ratios. Table 3 shows that in the presence of t^6, crossing-over between *T* and **T138** still occurs, indicating that the crossover-suppressing effect of t^6 does not extend throughout the chromosome. The *d*—**T138** recombination shows no significant difference in *T*+ and *Tt*^{6} heterozygotes ; so there is no evidence that

TABLE 3

Recombination of T *and* d *with the translocation break in* T138 *heterozygotes. All matings were backcrosses to* T138 T138 ++^{T}dd *animals*

Heterozygous parent	Phenotypes of progeny								Recombination			
	+T138*d*	*T*++	+T138+	*T*+*d*	*T* T138+	++*d*	*T* T138*d*	+++	T138-*T* OC	T138-*T* RC	T138-*d* OC	T138-*d* RC
+T138*d*/*T*++	12	7	4	2	1	...	6	5	25	12	30	7
T T138+/++*d*	1	...	5	2	8	8	2	4	22	8	23	7
						Total			47	20	53	14
						Recombination per cent.			29·9		20·9	
t^6 T138*d*/*T*++	15	11	6	1	1	...	1	3	33	5	30	8
T T138+/t^6+*d*	...	...	3	2	11	10	2	2	25	5	26	4
						Total			58	10	56	12
						Recombination per cent.			14·7		17·6	

OC = old combinations ; RC = recombinations.

the presence of t^6 alters crossing-over in arms of the translocation other than that in which T lies.

(iv) *The position of* T *and* tf *relative to the translocation break in* T138

A $T+$ *tftf* female was crossed with a **T138** **T138***dd* male, and $T+$ progeny were backcrossed to $++^T$ *tftf dd* animals. Since T shows approximately 30 per cent. crossing-over with the **T138** break and *tf* shows only 8·3 per cent. recombination with T, the order of loci could be expected to be either $T-tf-$**T138** break $-d$ or $tf-T-$**T138** break $-d$. The two possibilities could be differentiated by the fact that the least frequent classes of offspring could be taken to represent double crossover types. In this case the least frequent classes are $+tfd$ and $T++$ (table 4). These will be double crossovers if the order of loci is $T-tf-$**T138** break $-d$ but only single crossovers if the order is $tf-T-$**T138** break $-d$. The order is therefore shown to be $T-tf-$**T138** break $-d$. This was confirmed by tests of female progeny for **T138**; there were no animals in the classes $+tf$ **T138** and $T++$, which represent double crossovers if the order is $T-tf-$**T138** break $-d$.

One unexpected feature of the results is that the $T-tf$ recombination is enhanced in both sexes by the presence of the translocation. The observed recombination was $16{\cdot}7\pm2{\cdot}9$ per cent. in comparison with the $8{\cdot}3\pm1{\cdot}5$ per cent. previously observed in $T+\ +tf$ heterozygotes.

(v) *Crossing-over between* t^6 *and the translocation break in translocation* T190

Carter, Lyon and Phillips (1955) showed that translocation **T190** is closely linked to t^6 and reported one doubtful crossover between **T190** and t^6 in 102 tested animals. Further breeding has resulted in the discovery of two definite crossovers, one of the type **T190** $T/++$, which arose from a cross of a **T190** $t^6/+T$ male by a wild-type female, and one of the type $+^{T190}\ t/++$, which arose from a cross of a **T190** $t^6/+T$ female by a wild-type male. This latter crossover was of great interest since it proved to be a $t-$ mutant as well as a crossover. A descendant of the original crossover animal, believed to be of the genotype $+t^6$, was given to Professor Grüneberg. Crosses made by Professor Grüneberg of this animal to Tt^6 showed it to be in fact homozygous for a viable *t*-allele, for which the symbol t^{21} is proposed. The evidence for the genotype of this animal is as follows:

Parent mating	Progeny		
	Short tailed	Tailless	Normal
$t^{21}\ t^{21}$ ♀ × Tt^6 ♂	...	12	20
$t^6\ t^{21}$ ♀ × Tt^{21} ♂	1 ?	80	73

TABLE 4

Recombination of T *and* tf *with* d *and* T138 *in* T138 *heterozygotes.*
All matings were backcrosses to $++^{T}$ tfff $++^{T138}$dd *animals*

Heterozygous parent Type	Sex	Phenotypes of progeny							
		Tf+	++*d*	*T*+*d*	+*tf*+	*Tfd*	+++	+*tfd*	*T*++	Total
Tf++/++ T138*d*	♀♂	37	33	9	9	10	17	2	1	118
		13	13	3	2	8	4	1	…	44
				OC	RC	Total recombination per cent.				S.E.
	T-tf ♀			97	21	118		17·8		
	♂			38	6	44		13·6		
	♀ and ♂			135	27	162		16·7		2·9
	T-d ♀			73	45	118		38·1		
	♂			27	17	44		38·6		
	♀ and ♂			100	62	162		38·3		3·8
	tf-d ♀			88	30	118		25·4		
	♂			31	13	44		29·5		
	♀ and ♂			119	43	162		26·5		3·5
		Tf+	++T138	*Tf* T138	+++	*T*+T138	+*tf*+	*T*++	+*tf* T138	
Tf++/++ T138*d*	♀ and ♂	27	19	1	5	3	1	…	…	

If the female in the first cross had been $+t^6$ as expected, then the second crosses would have been $+t^6 \times Tt^6$ and in both types of cross short-tailed offspring should have been found,

In linkage tests with **T190** $T/++$ heterozygotes derived from the first crossover animal the number of recombinants was two out of fifteen animals tested, indicating that t^6 had, as supposed, been suppressing crossing-over between the **T190** break and T.

3. DISCUSSION

It has been shown that t^6 suppresses crossing-over in the $T-tf$ region. But t^6 had previously been shown not to differ from t^0, and t^0 had been shown to suppress crossing-over between T and Fu^{ki}. Dunn (1958) has recently shown, what was not known at the start of this experiment, that tf lies on the same side of T as Fu^{ki} and very close to Fu^{ki}. Hence it is still unknown whether the crossover-suppressing effect of t^6 extends on both sides of T. The effect of **T138** heterozygotes, where the presence of t^6 reduces $T-d$ recombination from 42 per cent. to 22 per cent. in females, suggests at first that the crossover-suppressing action extends further than had previously been known. However, the observation of enhanced crossing-over between T and tf in **T138** heterozygotes suggests that the region of crossover suppression is in fact quite short in the normal animal but that the translocation, by altering the chiasma localisation in the region, has caused an increased frequency of crossing-over in **T138** heterozygotes not carrying t^6.

An interesting and unexpected observation was the occurrence of rare crossovers in $+t^6$ $+tf$ heterozygotes and in **T190** heterozygotes. In the $T-tf$ region the observed recombination was 0/364 in Tt^6 $+tf$ heterozygotes and 2/162 in $+t^6$ $+tf$ heterozygotes, giving a combined total of 2/526. The frequency of mutants in balanced lethal lines carrying t-alleles is 1/500 (Dunn, 1956). Crossing-over in the $T-tf$ region in t-heterozygotes is thus of the same order of frequency as that of the mutation of these alleles. Furthermore, three of the four crossovers found in t-heterozygotes were of the type carrying the T or $+$ allele; the only one of the complementary type, that in **T190** which carried the t-allele, was found to carry a mutant t. Two explanations for such an occurrence are possible. First, that the presence of a t-allele induced a mutational change in the homologous chromosome, carrying tf, and that no true crossing-over occurred; and second, that the phenotypic and genetic effects of t-alleles are due to an abnormal chromosome segment in which rare crossing-over occurs and, by changing the length or position of the abnormal region, causes a change in the properties of the t-allele which is observed as a mutation. The fact that the mutation rate in balanced lethal t-stocks is so much higher than the spontaneous mutation rate at other mouse loci, and at the same time comparable with the recombination frequency in this chromosome region lends support to the second hypothesis. Such a hypothesis has been put forward previously by Grüneberg (1952).

Although the present data lend support to the hypothesis that mutation of *t*-alleles is in fact a result of crossing-over in an abnormal chromosome region, they are clearly insufficient to establish this point. It is important that more animals of the critical crossover type should be found ; it is hoped that an experiment now in progress may yield some of these animals as a by-product. The experiment is intended to find the effect of radiation on mutation in a Tt^6 balanced lethal stock. The stock carries *tf* heterozygously and hence tests of the *tf* genotype of mutant animals will show whether or not crossing-over has occurred simultaneously.

The hypothesis leads to the expectation that mutant *t*-alleles derived from the original t^0 and t^1 stocks still carry some part of the original chromosomal aberration and this may be expected to have some effect on crossing-over in their region, even if they are not complete crossover-suppressors. It is interesting in this connection that t^{13}, a mutant derived from t^1, appears to cause a sex-difference in crossing-over not usually found in this region. A further expectation is that the rare *T* crossovers found among the progeny of Tt^6 $+tf$ heterozygotes should carry some part of the abnormal chromosome region and should show some of the properties of *t*-alleles. Unfortunately, the crossovers of this type found so far have not been kept.

The original aim of these experiments was to find a crossover-suppressor suitable for use in mutagenetic studies. This aim has not been realised since the chromosomal region in which crossing-over is known to be suppressed by t^6 still remains short. Nevertheless, the new information gained about crossing-over in t^6 and t^{13} heterozygotes is such as to open up a new approach to the problem of crossing-over in mice carrying *t*-alleles.

4. SUMMARY

The experiments were aimed at extending knowledge of the crossover-suppressing effect of *t*-alleles in the hope that they might prove useful in making special stocks for studies in mutagenesis.

The lethal allele t^6 suppresses, almost but not quite completely, crossing-over between brachyury, *T*, and the recently found locus of tufted, *tf*.

The allele t^{13}, which is not lethal when homozygous, does not suppress crossing-over in this region, but in Tt^{13} $+tf$ heterozygotes there is a sex-difference in crossing-over which is not seen in $T+$ $+tf$ heterozygotes.

Tufted was found to lie between *T* and the **T138** translocation break, and the $T-tf$ recombination in **T138** heterozygotes was enhanced. t^6 reduces crossing-over between *T* and the translocation break in translocation **T138**, and the amount of reduction is such as to make it appear that the crossover-suppressing effect extends little, if at all, beyond *tf*. The effect of t^6 on crossing-over on the side of *T* remote from *tf* is still unknown.

The frequency of the rare crossovers in the progeny of t^6 —*tf* heterozygotes is of the same order as that of the mutant *t*-alleles found in balanced lethal *t*-stocks. Moreover, one of the rare crossovers found between t^6 and the translocation break in **T190** was discovered to be a mutant as well as an apparent crossover. In view of these two facts the possibility is discussed that the high rate of mutation in balanced lethal *t*-stocks may in fact be due to crossing-over in an abnormal chromosome region.

Acknowledgments.—The authors are grateful to Professor L. C. Dunn for his interest and for the gift of mice carrying t^{13}, and to Miss M. L. Court for technical assistance.

5. REFERENCES

CARTER, T. C., LYON, M. F., AND PHILLIPS, R. J. S. 1955. Gene-tagged chromosome translocations in eleven stocks of mice. *J. Genet.*, *53*, 154-166.

CARTER, T. C., LYON, M. F., AND PHILLIPS, R. J. S. 1956. Further genetic studies of eleven translocations in the mouse. *J. Genet.*, *54*, 462-473.

CARTER, T. C., AND PHILLIPS, R. J. S. 1950. Three recurrences of mutants in the house mouse. *J. Hered.*, *41*, 252.

DUNN, L. C. 1956. Analysis of a complex gene in the house mouse. *Cold Spring Harbor Symp.*, *21*, 187-195.

DUNN, L. C. 1958. Private communication : *Mouse News Letter No. 18.*

DUNN, L. C., AND CASPARI, E. 1945. A case of neighbouring loci with similar effects. *Genetics*, *30*, 543-568.

DUNN, L. C., AND GLUECKSOHN-SCHOENHEIMER, S. 1950. Repeated mutations in one area of a mouse chromosome. *Proc. Nat. Acad. Sci.*, *36*, 233-237.

DUNN, L. C., AND GLUECKSOHN-WAELSCH, S. 1953*a*. Genetic analysis of seven newly discovered mutant alleles at locus *T* in the house mouse. *Genetics*, *38*, 261-271.

DUNN, L. C., AND GLUECKSOHN-WAELSCH, S. 1953*b*. The failure of a *t*-allele (t^2) to suppress crossing-over in the mouse. *Genetics*, *38*, 512-517.

GRÜNEBERG, H. 1952. *The Genetics of the Mouse*, 2nd edition. The Hague : Martinus Nijhoff.

LYON, M. F. 1956. Hereditary hair loss in the tufted mutant of the house mouse. *J. Hered.*, *47*, 101-103.

PHYSIOLOGICAL GENETICS OF *PISUM II.*

THE GENETICS OF PHOTOPERIODISM AND VERNALISATION

H. N. BARBER
University of Tasmania

Received 1.v.58

1. INTRODUCTION

THE genetics of photoperiodism or vernalisation has been investigated in several different species of plants (Lang, 1948). Rarely has it been possible to investigate the inheritance of both types of behaviour in the one series of crosses. This has meant that the relationship between the two patterns of behaviour has had to be worked out solely by the methods of experimental physiology. The physiologists' approach has led to the idea of a phasic development in those plants showing both types of behaviour. This idea was expressed in its crudest form by Lysenko (Whyte, 1948) and has been developed, particularly by Gregory (1948) working on cereals and Melchers and Lang (1948) using *Hyoscyamus*, into the concept that a period of growth at low temperatures (vernalisation) is necessary to provide the substrate ("vernalin") from which the flowering hormone ("florigen") is manufactured under the correct photoperiod.

If this idea of a chain of reactions each link of which is sensitive to a different environmental factor, and in which the whole chain leads to the synthesis of a positive flowering hormone, is of universal validity, it should be possible to isolate genes controlling the separate reactions. In different species allelic genes or polygenic systems are known controlling the following differences :—(*a*) vernalisation required : vernalisation not required ; (*b*) long-day : photoperiodically indeterminate ; (*c*) short-day : photoperiodically indeterminate. Segregation of genes controlling other possible differences in patterns of behaviour has not been realised nor has it been possible accurately to study genetic segregation for both vernalisation and photoperiodism in the one species. Attempts to isolate genes controlling both types of differences have been made in sugar beet (Owen *et al.*, 1940) and subterranean clover (Aitken, 1955, Davern *et al.*, 1957). The curious situation has arisen in both plants that segregation affecting differences in flowering behaviour affects both reactions simultaneously. Although analysis in both species is incomplete, the difficulty in separating the two reactions genetically might lead one to deduce that both types of difference in behaviour are functions of the one pair of alleles or of the one polygenic system. This situation is, perhaps, unexpected on the Gregory-Melchers scheme.

There are also indications at the physiological level that the idea of phasic development is not of universal application. Aitken, in particular, has described in subterranean clover a situation which is

best described as a competition between cold requirement and light, prior vernalisation reducing the photoperiodic response. This pattern of behaviour may be widespread although the experimental evidence is seldom sufficient to prove it (*e.g.* Owen's " photothermal " control in sugar beet, Thompson's (1953) horticultural studies on radishes, cabbages, celery, etc.).

There is another point at issue. It has become the fashion in some circles to discuss flowering almost entirely as though its control was by way of the formation of stimulating substances at definite periods during the development of the plant. Gregory mentions in passing that perhaps we should more often think in terms of removal of inhibitors. Hamner (1948) also discusses this general problem but decides there is little need to postulate inhibitors. Harder (1948) and von Denffer (1950) have advocated the view that inhibitors are active in the control of flowering. Von Denffer has suggested in this connection that we may have to add another function to the already numerous functional roles of auxin.

There is little conclusive evidence either way. There is good experimental evidence to show that a small region of the plant may be given a treatment which then makes the treated region capable of inducing flowers in remote parts of the plant. The induction of a single leaf by photoperiod or the grafting in of a vernalised apex are examples of this action-at-a-distance. It is natural to explain such a situation by postulating that the region inducing flowering is the source of a stimulator which is then transported to the apical regions which respond by the production of flowers. However, the experimental facts can often equally well be described in terms of the regions capable of inducing flowering acting as " sinks " for the destruction of hormonal substances inhibiting flowering. It seems time that some formal word to describe this functional class of substance be introduced. I therefore suggest that hormonal substances with the property of delaying or inhibiting the production of flowers be referred to as " *colysanthins* ".*

It is impossible to determine how many of the presumptive " sources " of florigen or vernalin are really " sinks " for colysanthin. It is known that the interpolation of non-photo-induced tissue between an apex capable of flowering and the induced tissue, may nullify the effects of the induced tissue on the apex (Harder, 1948). This fact seems more easily interpretable on the idea of a " sink " removing a colysanthin than on the formation and subsequent destruction of a florigen. It is dangerous to rely, with Stout (1945) and Bonner and Galston (1952) on one theory of phlœm transport to explain such effects.

It is, perhaps, pertinent to remark here that in their classical paper of 1938, Bonner and Hamner remark : " Two hundred and forty-six

* I am indebted to Professor J. R. Elliott for this suggestion. Colysanthin is derived from Κωλυω and *ἀνθος*—to delay flowers. I hesitate to suggest the abolition of the word " florigen " ; but Professor Elliott feels that *anthophorin* or *anthogen* would be more comfortable words.

different kinds of extracts and extract fractions were made during the course of attempts to extract the floral initiation substance from leaves." They also tested between twenty and thirty pure compounds including vitamins, amino acids, plant acids, etc. They conclude : " There are indications, however, that if the vegetative cuttings are treated by slight modifications of the procedure here outlined, they may respond with the initiation of floral primordia to the application of a simple water extract of leaves from induced *Xanthium* plants."

An enormous effort has continued along these lines during the twenty years since publication of their paper. Florigen has still to be isolated and interest has naturally shifted to the study of ever more complex and labile fractions (*cf.* Finch and Carr, 1956, on nucleic acid fractions). Highkin (1956) has given a preliminary report suggesting that certain naturally occurring purine derivatives can accelerate flowering in peas. There have also been accounts in recent years suggesting that a variety of pure substances can accelerate flowering in the following ways :

(*a*) By removing the requirement for vernalisation (Lang, 1956 ; gibberellin on biennial *Hyoscyamus*, *Daucus*, etc.),

(*b*) By partially or wholly removing the photoperiodic requirement in long-day plants (Liverman and Lang (1956) on *Hyoscyamus* and *Silene* by indoleacetic acid ; Melchers and Lang (1942) on *Hyoscyamus* by sugars ; Langridge (1957) on *Arabidopsis*, Bunsow and Harder (1956) on *Lapsana*, Lang (1956) on *Samolus*, etc., by gibberellins),

(*c*) By some unspecified action on plants with a specific qualitative or quantitative vernalisation or photoperiodic requirement. (Highkin (1956) by guanosine on peas, Paton (unpublished) on peas and Wittwer *et al.* (1954) on celery by maleic hydrazide),

(*d*) By some unspecified action on plants with an indeterminate pattern of flowering (Clark and Kerns, 1942, van Overbeek, 1946, on pineapples, Shigeura, 1948, on the litchi, Howell and Wittwer, 1954, on the sweet potato, all of whom used indoleacetic acid).

Examples of chemical inhibition are fewer. Gibberellic acid delays flowering in early and late varieties of peas (Barber *et al.*, 1958). Harder and Bunsow (1957) have shown that gibberellic acid prevents flowering in the short-day plant *Kalanchoe* when it is applied to leaves receiving photo-inductive treatment. Indoleacetic acid will also destroy photo-induction of short-day plants under certain conditions (Bonner, 1948, on *Xanthium*). Haupt (1952, 1957) has demonstrated that yeast extract will delay flowering in excised pea embryos grown on a synthetic medium under short days. In darkness or continuous light the delay is much less.

Examples of non-chemically-defined inhibitions are more numerous, *e.g.* the non-induced leaves mentioned above ; the fact that removal of the leaves of vernalised *Hyoscyamus* will allow the plant to flower in short days (Lang and Melchers, 1943) ; Guttridge's (1956) postulation of a stimulus promoting vegetative as opposed to reproductive growth

in strawberries; Tashima's (1953) demonstration that the leaves of radish grown on a synthetic medium in short days delay flowering by 2-3 nodes as compared with total darkness or continuous light; Lockhart and Hamner's (1954) demonstration that a second dark period immediately following an inductive dark period in *Xanthium* reduces or inhibits flowering (*cf.* also Skok and Scully, 1954); Sprent and Barber's (1957) demonstration that soaking in water of young cuttings of late varieties of peas results in earlier flowering. There are few if any descriptions of experiments designed to test the inhibiting influence of grafted vegetative apices on the flowering of apices which are grown under conditions favourable to flowering.

It is still impossible to say whether any of the chemical treatments can be said to duplicate the normal action of any particular genotype in any particular environment. To answer this question it is necessary to demonstrate that the added chemical substance is a normal plant constituent which is produced by the genotype when, and only when, an environment appropriate for flowering is provided for that genotype. Proofs of this nature are possible by the ordinary methods used by the typical physiologist. They may, however, be facilitated by using as experimental material populations of plants differing by one or more genetically definable differences in pattern of flowering. Barber and Paton (1952 and 1955) by using such differences concluded that genetically late varieties of peas produced a colysanthin which could pass a graft union and delay the flowering of genetically early scions. The existence of a florigen was not excluded. The late varieties of peas are also vernalisable and photoperiodic whilst the earlies are non-vernalisable and non-photoperiodic. Further grafting experiments (Barber and Paton, unpublished) have shown that a vernalised rootstock of a late variety caused less delay in the flowering of an early scion than an unvernalised stock and that a leafy late stock will delay flowering in an early scion under short days but not under long days.

This paper gives an account of the responses to photoperiod and to vernalisation of pure varieties, F_1 and F_2 hybrids. It has so far proved impossible to separate either genetically or physiologically the two reactions. Both reactions seem to be controlled by the one major gene, *Sn*, and a competitive relation exists between the two behaviour patterns. In addition to its effect on flowering the *Sn* gene has several other pleiotropic effects. An attempt has also been made to observe pleiotropic effects on flowering of other segregating genes, *e.g.* at the *Le* locus governing internode length.

2. MATERIALS AND METHODS

For the crossing programme the four varieties, Alaska (tall, early), Massey (dwarf, early), Telephone (tall, late) and Greenfeast (dwarf, late) were chosen. F_1 and F_2 seed was raised in Hobart and flown to the Earhart Laboratory, California Institute of Technology, where it was grown under controlled environments. A few tests were made using the varieties Zelka and Unica. Vernalisation treatments involved a period of three weeks at 4° C. immediately after soaking the seed.

The plumule was usually less than 1 cm. in length at the conclusion of the treatment. Photoperiodic treatment usually involved a period of 8 hours under natural illumination. This period was then supplemented by artificial fluorescent and incandescent light at 500-1000 foot-candles. All photoperiods were on a cycle of 24 hours. Temperature during growth was usually 17° C. In addition a few experiments were performed using artificial light entirely. However, at short photoperiods (8 or 12 hours) there was a tendency for loss of apical dominance making scoring impossible. Some experiments were also conducted at different growing temperatures (10° C., 17° C., 23° C.).

Cultural treatment was described in Went (1957), the plants being grown in a mixture of vermiculite and gravel and watered with a full culture solution. No nodulation occurred. In general a factorial experimental design was followed since this design allows the interactions of the different treatments to be most easily and accurately estimated. For the pure varieties twelve or sixteen replications were made. Mortality was low, the principal cause of loss being accidental decapitation. Where unequal numbers survived, plants were discarded at random to preserve the symmetry of the experiment and the analysis of variance.

Plants were scored for node at which the first flower was initiated, node of first leaf with more than two leaflets, length of stem between nodes 5 and 9, etc. The cotyledonary node was excluded. At short photoperiods and high temperatures abortion of the first flowers initiated is frequent. However, there is never any chance of confusion of an aborted flower with a vegetative axillary bud. Early abortion may result in the bracteoles of the flower hypertrophying into peculiar almost connate structures up to 0·5 cm. in diameter. Once a flower is initiated reversion to a vegetative axillary at higher nodes is very rare if the environmental cycle is maintained unchanged. Out of several thousand plants only two cases of reversion were noticed under these conditions. Photoperiodic induction is, however, reversible during the early period of development (see below).

3. RESPONSE OF PURE VARIETIES TO VERNALISATION AND PHOTOPERIOD

(a) *Node of initiation of first flower (F)*

The behaviour of the four varieties Alaska, Massey, Greenfeast and Telephone and the F_1, Telephone × Massey, are illustrated in figs. 1 and 2 which are based on the data given in table 1. The graphs give the results of factorially designed experiments based on the combination of two temperature treatments (no vernalisation, UV, vernalisation, V) with three photoperiods (8 hours natural daylight, and 16 hours darkness, P_8, 8 hours daylight and 4 hours artificial light and 12 hours darkness, P_{12}, and 8 hours daylight and 16 hours artificial, P_{24}). The temperature at which the plants were grown was 17° C. night and day. There were ten or twenty replications in each variety but only five for the F_1. Error estimates are given in table 1.*

The varieties fall into two sharply separated groups. The early varieties flowering at node 9 or 10 show no response to photoperiod, Alaska also showing no response to vernalisation. Massey shows a slight but significant *negative* vernalisation, vernalised plants flowering

* Table 5 shows that there are very considerable changes in variance in the different environments, flowering becoming more variable in those environments unfavourable to it. In view of the size of environmental effects, it is clear that in most if not all cases the changes in variance would not alter the statistical significance of the effects of environmental variables and their interactions.

0·5-1 node later. The effect is highly significant statistically ($P < 0.001$). The apparent reduction in this negative vernalisation with long photoperiod is not statistically significant ($P > 0.2$). The negative

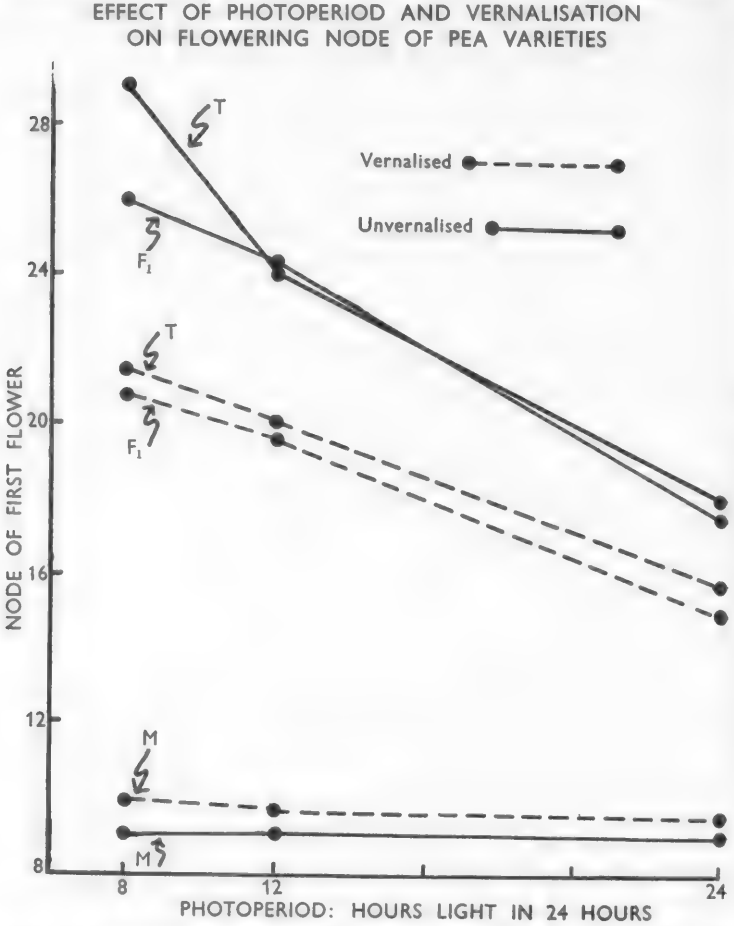


FIG. 1.—Graphs giving effect on node of first flower of factorially combined vernalisation and photoperiodic treatments for the varieties Massey (M) and Telephone (T) and their F_1. Number of replications and standard errors of estimates of treatment means are given in table 1.

vernalisation of Massey is similar to the results of Leopold and Guernsey (1954) on the variety Alaska where they obtained evidence of an optimum temperature between 18 and 22° C. following short (5-day) temperature treatments. Haupt (1952) using the early variety "Kleine Rheinländerin" could obtain no response to either photoperiod or temperature.

The late varieties and the F_1 are both vernalisable and quantitative long-day plants, increasing photoperiod lowering the node of first flower apparently almost linearly in both vernalised and unvernalised plants. For unvernalised plants increasing the photoperiod from P_8 to P_{24} lowers the node of first flower 8-12 nodes in the different varieties. In vernalised plants this photoperiodic response is lower, being 5 to 7

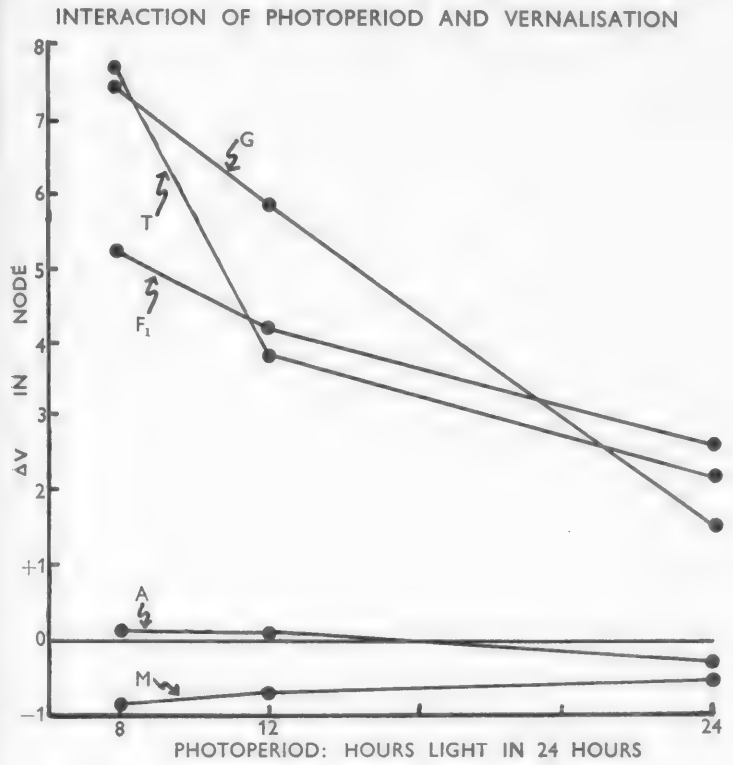


FIG. 2.—Dependence of response to vernalisation on photoperiod for varieties Massey, Telephone and their F_1 and varieties Greenfeast (G) and Alaska (A).

nodes. Correspondingly the response to vernalisation is greatest at the shortest photoperiods, falling from 5 to 8 nodes at P_8 to 1 to 3 nodes at P_{24} (fig. 2). These interactions are all very highly significant ($P<0{\cdot}001$).

Similar experiments using the varieties Zelka and Unica were also performed. Zelka with factorially arranged treatments of V, UV, P_8 P_{16} and P_{24}, is very similar in behaviour to the late varieties already discussed. Unica was tested under photoperiods of artificial light only. Growth was poor, most of the plants grown at P_8 dying before scoring was possible. Both V and P responses were smaller

than in the other varieties ($\triangle P = 0{\cdot}36$ node in UV plants grown at P_{12} and P_{24}, $\triangle V = 1{\cdot}54$ node at P_{12}) and there was no significant interaction of the P and V treatments.

A few experiments on the effect of temperature at which the plants were grown were also carried out using the varieties Massey, Telephone and Unica. Temperatures chosen were 10° C., 17° C. and

TABLE 1

Flowering response of pure varieties to vernalisation and photoperiod

The table gives mean node of first flower, F, for 6 treatments, combining 2 vernalisations (UV and V) and 3 photoperiods.

Variety	Vernalisation	Photoperiod				No. of replications	S.E. of treatment mean	Significance of PV interaction
		P_{24}	P_{16}	P_{12}	P_8			
Early								
Alaska	UV	10·0	...	10·2	10·2	10	0·120	N.S.
Massey	UV	9·1	...	9·1	9·1	10	0·111	N.S.
Alaska	V	10·3	...	10·2	10·1	10	0·120	...
Massey	V	9·6	...	9·8	10·0	10	0·111	...
Late								
Greenfeast	UV	16·5	...	25·7	(29·4)*	10	0·321	0·001
Telephone	UV	18·0	...	23·95	29·0	20	0·330	0·001
$F_1T \times M$	UV	17·6	...	24·0	26·0	5	0·270	0·001
Unica	UV	18·91	19·36	19·27	died†	11	0·275	N.S.
Zelka	UV	15·8	17·4	...	26·0	10	0·359	0·05-0·01
Greenfeast	V	14·9	...	19·8	(22·0)*	10	0·321	...
Telephone	V	15·8	...	20·1	21·35	20	0·330	...
$F_1T \times M$	V	15·0	...	19·8	20·8	5	0·270	...
Unica	V	16·82	17·09	17·73	died†	11	0·275	...
Zelka	V	13·6	14·1	...	21·7	10	0·359	...

* Only 7 and 6 plants survived to scoring date under P_8 for Greenfeast. S.E. of estimates of means are UV, P_8, 0·719 and V, P_8, 0·516 node.

† Unica was grown under artificial light only. Sixteen replicates planned reduced to 11 by deaths most of which were due to poor growth.

23° C. Illumination was wholly artificial and particularly at the higher temperatures and under short photoperiods growth was poor. Results for Telephone and Massey are given graphically in fig. 3. As might be expected, Massey shows only a small response. In unvernalised plants there is a delay in flowering of about one node at 10° C. as compared with higher temperatures. The effect is statistically significant ($P < 0{\cdot}01$). Prior vernalisation appears to remove the effect of growth-temperature.

Telephone and Unica show a greater response to growth-temperature. The size of the effect depends on both photoperiod and vernalisation. In both varieties prior vernalisation and long photoperiod reduce the effect of growth-temperature. In other words,

the lower the temperature of growth, the less the effect of vernalisation and of photoperiod.

The interaction of vernalisation and growth-temperature is to be

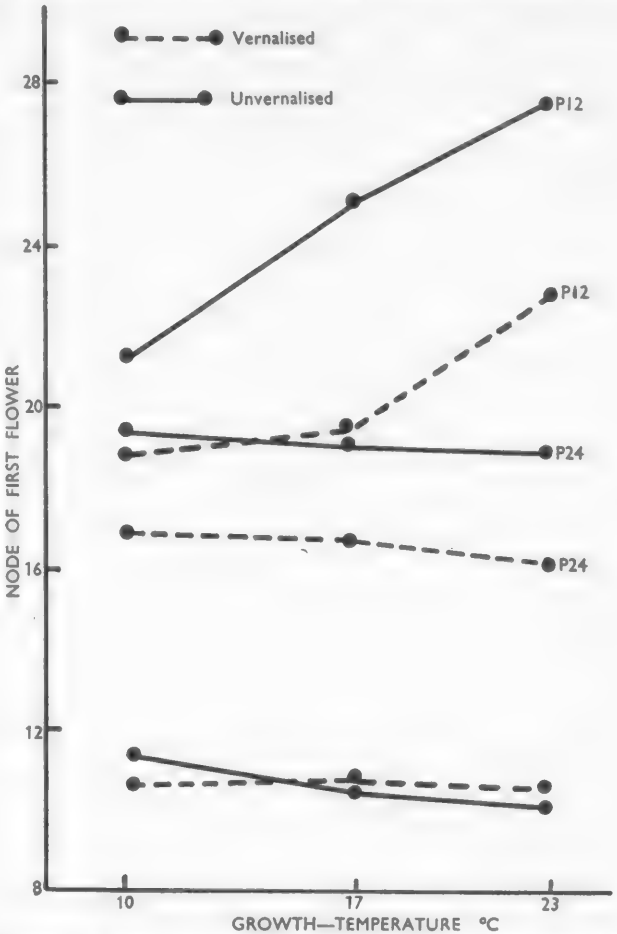


FIG. 3.—Effects of growth-temperature on node of first flower in the varieties Massey (lower curves) and Telephone (upper curves). In Massey photoperiod (P_{12} and P_{24}) has no effect on node of first flower. The data have therefore been grouped. In Telephone growth-temperature has no significant effect in continuous light (P_{24}). In photoperiods of 12 hours, the effect of growth-temperature is reduced by prior vernalisation.

expected. It presumably means that vernalisation in peas is a continuous process not restricted to the early stages of growth although as Highkin (1956) has shown the effect of temperature is most easily detected during the early stages of development. Both reactions seem

easily interpretable along the lines suggested by Melchers and Lang (1948), one of the biochemical reactions governing flowering having a lower temperature coefficient (Q_{10}) than the reactions promoting vegetative growth. Highkin has shown that vernalisation also reduces the growth in length of the stem and that this effect is not reversible by high temperatures applied immediately after vernalisation. The effect on node of first flower is reversible under these conditions.

To test whether the photoperiod induction of flowers is reversible, plants of the varieties Telephone and Zelka were grown under a photoperiod of 8 hours at a temperature of 20° C. Batches of twelve plants were given continuous light for seven days at successive weekly intervals after germination. The results were similar in both varieties. Owing to poor survival of Telephone, only the results on Zelka are given in detail in fig. 4. It will be seen that treatment of seedlings with continuous light for the first ten days after germination has no effect on node of initiation of the first flower. At the end of the period the seedlings had one foliage leaf fully expanded with the second leaf beginning to expand. Treatment with continuous light during the next week, during which time a further two leaves expanded, gave a fully effective photoperiodic response. These plants flowered at a mean node of 16·9 whilst plants grown throughout their life in continuous light flowered at a mean node of 16·3. Later exposures to a week of continuous light lead to a gradual raising of node of first flower towards the first flowering node of 38 obtained when plants are kept throughout at P_8.

As regards reversibility of photoperiodic induction, treatments 2, 3 and 4 all gave some plants which showed reversion to nodes producing vegetative axillary buds. In treatment 2, seven out of ten plants reverted, the mean node of reversion being 25·0. These plants produced 4·3 vegetative nodes and came into flower again at a mean node 29·3. There were one and two reversions in treatments 3 and 4. The behaviour of these plants was similar to those reverting after treatment 2 except that the node at which flowering first started was higher (node 19 after treatment 3, nodes 22 and 21 after treatment 4).

These results show that photoperiodic induction is completely reversible in late varieties of peas.

An interaction of photoperiod and vernalisation (or growth-temperature) in the sense observed in the late varieties of peas is unexpected on the Gregory-Melchers view of the relationship of these two processes. If the idea of a chain of reactions leading to the synthesis of a florigen or the removal of an inhibitor is adopted, prior vernalisation by providing the substrate for the photoperiodic reaction would be expected to enhance, not to decrease, the magnitude of the photoperiodic response. In peas the relationship between the two processes is competitive not complementary. In other words, a period of cold treatment partially replaces the necessity for exposure to long photoperiods.

How widespread this pattern of behaviour is, is still unknown. In only a few plants have the necessary experiments been done. The same pattern undoubtedly exists in *Trifolium subterraneum*, *Medicago* and other pasture legumes (Aitken, 1955). It probably exists in sugar

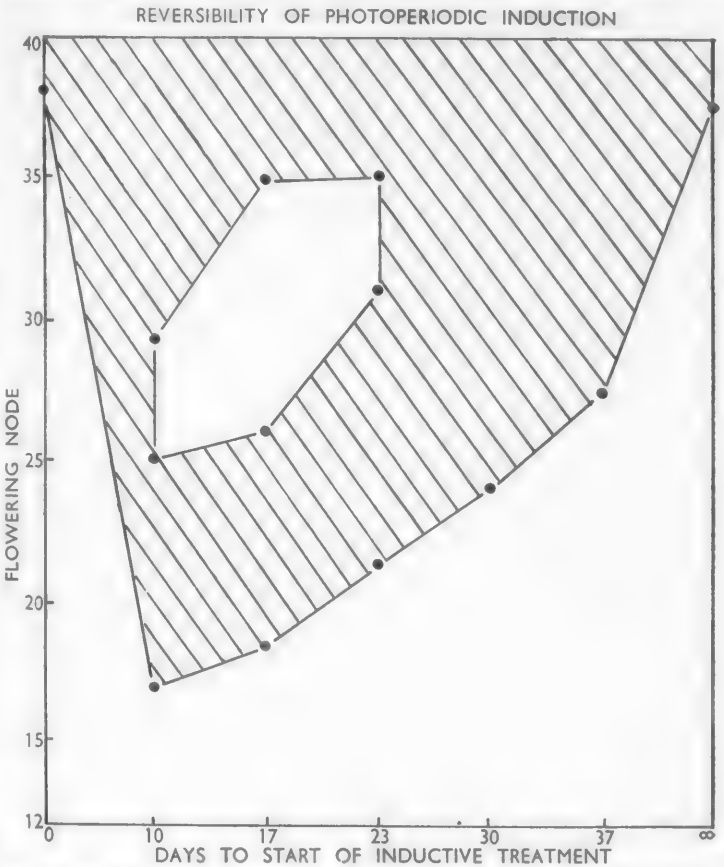


FIG. 4.—Diagram showing reversibility of photoperiodic induction. Plants grown in P_0 except for 1 week's continuous light. Abscissa gives time in days from start of germination to beginning of week's treatment with continuous light. Shaded area shows conditions and nodes where flowering occurs in all plants. Unshaded area outside curve gives non-flowering region. Unshaded area within curve gives mean behaviour of those plants which reverted to vegetative growth.

beet (*Beta vulgaris*, Owen *et al.*, 1940) and other related chenopods. If one reads between the lines of the many accounts of the experiments on vegetables reviewed by Thompson (1953), the same pattern may exist in Umbelliferæ, *e.g.*, celery, carrot, Cruciferæ, *e.g. Brassica*, *Raphanus*, etc.

It is, perhaps, worth discussing the possible adaptive value of this competitive (or replacement) pattern of the control of flowering as compared with the more popular and older idea of a phasic development. Phasic development assumes that a chain of reactions must occur in a set order before the plant can flower. Such a chain is obviously much less likely to be buffered against normal environmental fluctuations than is the replacement pattern. On the hypothesis of phasic development, as particularised by Melchers and Gregory, we can predict that a winter giving maximum vernalisation will give the most sensitive photoperiodic response. A warm winter may be followed by a weaker photoperiodic response. Any variation in degree of the completion of the first reaction of the chain may tend to be magnified by the subsequent reactions. Functionally, this seems a dangerous state of affairs capable of leading to an erratic control of time of flowering. The replacement pattern, on the other hand, is self-buffering. Two mechanisms have been developed by the plant either or both of which may give a time of flowering sufficiently stable for survival.

(b) *Node of formation of first leaf with more than two leaflets (C)*

The first formed foliage leaves of most varieties of peas have two large foliose stipules, two opposite pinnæ and then several pairs of opposite tendrils. Later in development a pair of tendrils is replaced by another pair of pinnæ to give a leaf with four leaflets. In some varieties a further pair of tendrils may be replaced by leaflets at nodes higher up the stem. The replacement of the tendrils is usually symmetrical but sometimes one-sided replacement does occur and there may be occasional reversion from a higher to a lower number of leaflets.

There is evidence that this pattern of leaf development is to some extent correlated with the flowering behaviour. The change to a four-leaflet leaf occurs at a higher node in the late-flowering varieties. The development of the leaf-lamina is to some extent controlled by purine derivatives (Bonner *et al.*, 1939). In view of these facts and of Highkin's suggestion that treatment with purine derivatives may control the position of the first flower, it seemed worthwhile to investigate, concurrently with flowering node, the effect of photoperiod and vernalisation on the node at which the transition from two to more than two leaflets first occurred. This node will be designated by C.

The results are given in table 2 (*a*). Photoperiod has apparently little if any effect on C in Telephone and Massey and their F_1. There is a small effect which is statistically significant in Alaska and Greenfeast. In these last two varieties reducing the photoperiod from P_{24} to P_{12} delays the production of leaves with more than two leaflets by 0·5-1 node. Vernalisation significantly alters C in all varieties except Alaska. In the late varieties, vernalisation reduces C by 1·2

nodes. In Massey vernalisation increases C. There is, thus, a parallelism in reaction to vernalisation in both flowering node and C.

TABLES 2 (*a*) and (*b*)

Vegetative responses of pure varieties to vernalisation and photoperiod

Table 2 (*a*) gives mean node, C, at which first leaf with more than 4 leaflets is produced in 6 treatments combining 2 vernalisations (UV and V) and 3 photoperiods. Table 2 (*b*) gives similar analysis for L in cm., the length of stem between nodes 5 and 9. Number of replications as in table 1. Since interactions are usually not significant, in both tables grouped means have been given.

TABLE 2 (*a*)

Variety	S.E. of treatment mean	Photoperiod			Significance	Vernalisation		Significance	Significance of PV interaction
		P_{24}	P_{12}	P_8		UV	V		
Early									
Alaska .	0·156	9·10	9·50	9·45	0·05	9·37	9·33	N.S.	N.S.
Massey .	0·135	10·10	10·10	9·95	N.S.	9·90	10·20	0·01	N.S.
Late									
Greenfeast	0·270	11·05	12·35	(12·70)*	0·001	12·55	10·70	0·001	N.S.
Telephone	0·354	16·00	15·68	16·15	N.S.	16·27	15·62	0·05	0·05
F_1 T×M .	0·698	12·80	14·40	14·00	N.S.	14·53	12·93	0·01	N.S.

TABLE 2 (*b*)

Early									
Alaska .	0·606	32·35	26·95	21·90	0·001	26·40	27·70	N.S.	0·001
Massey .	0·384	12·70	10·85	9·65	0·001	13·20	8·90	0·001	N.S.
Late									
Greenfeast	0·167	6·35	6·10	(4·96)*	N.S.	7·35	5·10	0·001	N.S.
Telephone	0·654	26·48	15·80	15·18	0·001	21·90	16·40	0·001	N.S.
F_1 T×M .	1·342	26·00	20·50	16·80	0·001	25·80	16·40	0·001	N.S.

* Owing to poor survival of plants at P_8, full analysis of variance not possible for Greenfeast. S.E. of estimates of means of survivors under P_8 are : for C, 0·411 node and for L, 0·665 cm.

Where a reaction to photoperiod occurs it is also in the same sense as the reaction of the flowering node. The effects on C are smaller and there is not the strong competitive interaction between response to photoperiod and vernalisation. The absence of interaction has allowed the grouping of means in table 2 (*a*).

(c) *Length of stem between nodes 5 and 9 (L)*

As mentioned earlier, there have been numerous claims that auxins may affect the pattern of flowering. Gibberellic acid has been shown by several authors to affect flowering in several species of plants. Brian and Hemming (1955) have also shown that gibberellic

acid treatment will enable the recessive dwarf pea, *le*, to produce a phenocopy of the tall, *Le*. Thus, it seemed worthwhile to investigate the effects of photoperiod and vernalisation on growth in length of the stem. The distance, L, between node 5 and 9 was chosen because most of these nodes are laid down after germination and before flowering. The onset of flowering usually causes a sudden decrease in growth in length of internodes.

The results are shown in table 2 (*b*) which shows that both photoperiod and vernalisation have highly significant effects on length of the stem. In contrast to the effects on flowering there is no significant interaction. The effects are simply additive. For all varieties the shorter the photoperiod, the shorter the stem. There is some suggestion that plants of the *Le* phenotype are affected proportionally more than those of *le* phenotype. In Greenfeast and Massey reducing photoperiod from P_{24} to P_8 reduces stem length by 21·9 per cent. and 23·7 per cent. respectively whilst for the tall varieties, Telephone, Alaska and the F_1 of Telephone and Massey, the reductions are 42·5 per cent., 35·4 per cent. and 32·3 per cent.

Vernalisation, with the exception of Alaska, also reduces stem length. The reductions here seem to be the same whatever the genotype or phenotype with regard to the *Le* locus. They range from 25·1 per cent. to 36·0 per cent.

The data are scarcely extensive enough to show what the effect might be of substituting genes controlling late flowering for alleles controlling early flowering with regard to these growth patterns. The reactions appear to be the same in both early and late varieties. There is no explanation of the anomalous behaviour of the Alaska towards vernalisation.

4. GENETIC SEGREGATION UNDER CONTROLLED CONDITIONS

We have seen that the F_1 from Massey ×Telephone behaves in all respects like the late parent Telephone, *i.e.* the late-flowering habit is dominant. Similar crosses of late ×early have been made using Alaska ×Telephone, Greenfeast ×Massey, and Alaska ×Greenfeast. In these cases the F_1 were grown only at Hobart. But in all cases the mean flowering node of the F_1 was very close to the late parent. In three of the four cases, the F_1 flowers at a slightly (0·25-0·75) lower node than the late parent grown under the same conditions. No reciprocal differences were noted.

F_2 were also raised at Hobart in the garden in summer and under the short-day conditions of the winter in a heated glasshouse with large gradients in temperature and light intensity. In neither case was a discontinuous segregation in type of flowering behaviour obtained. In summer the segregations were usually bimodal and could be interpreted as showing both a segregation of a major gene giving three late : one early and associated modifiers and polygenes (*cf.* Tedin, 1923, and Pellew, 1940). There is at least a 10 per cent. error possible in

classification solely on phenotypes. Winter sowings of smaller numbers of plants gave a continuous unimodal segregation. The difference between summer and winter sowings showed that genes controlling

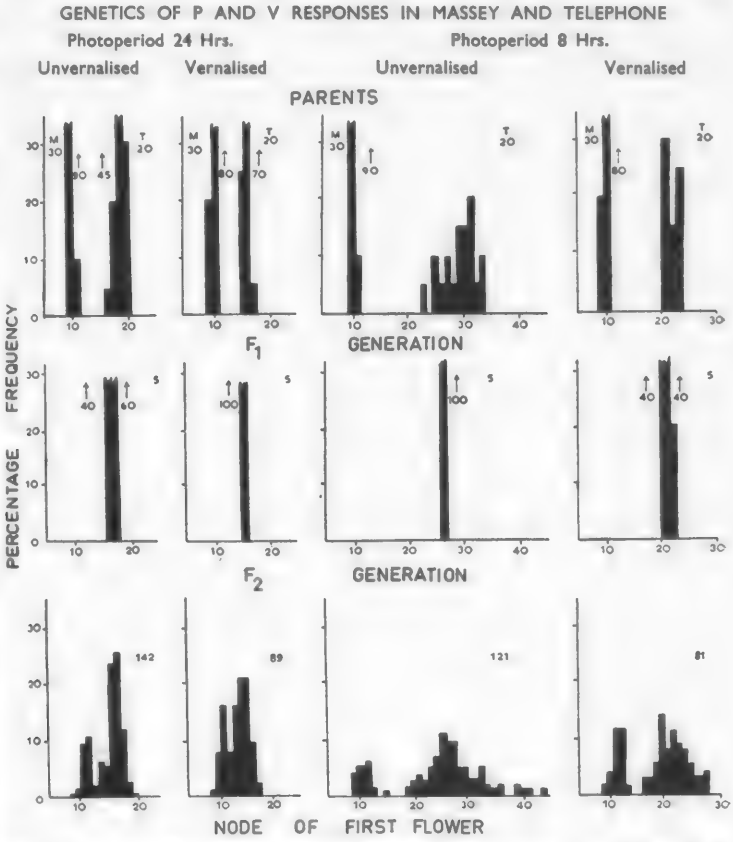


FIG. 5.—Histograms showing behaviour of Massey and Telephone, their F_1 and F_2 when grown under the four environments: continuous light, unvernalised and vernalised, and photoperiod of 8 hours, unvernalised and vernalised. Number of plants in samples given in top right and left hand corners of histograms.

the development of vernalisation and photoperiodic reactions were segregating in F_2.

In order to clarify the genetic system further, parental, F_1 and F_2 seed were sown in the constant environments of the Earhart Plant Research Laboratory, California Institute of Technology. The data are given in fig 5. and table 3. The cross Massey ×Telephone gave the fullest data and will be dealt with first. In this cross the *Le* : *le* pair of alleles which control internode length, is also segregating. It

will be seen that discontinuous segregations with regard to node of first flower are obtained when the F_2 is grown under short days. Under

TABLE 3

Genetic segregations in F_2

Gross environment		Phenotypes					Total planted	χ^2
P	V	*Le, Sn*	*Le, sn*	*le, Sn*	*le, sn*	T		
M×T (homozygous for *r*)								
24	UV	70	30	39	5	144	144	...
12	UV	87	27	19	7	140	144	...
8	UV	80	25	23	4	132	144	...
Totals . . .	*UV*	*237*	*82*	*81*	*16*	*415*	*432*	4·21
24	V	(65 *Le*		(24 *le*)		89	92	...
12	V	41	19	16	4	80	92	...
8	V	45	20	18	3	86	92	...
Totals . . .	*V*	*86*	*39*	*34*	*7*	*166*	*184*	3·95
Grand Totals . .	*UV+V*	*323*	*121*	*115*	*23*	*582*	*616*	6·61
		Sn, R	*Sn, r*	*sn, R*	*sn, r*			
A×T (homozygous for *Le*)								
24	UV	(52 *R*)		(24 *r*)		68	76	...
12	UV	33	19	12	4	68	76	...
8	UV	33	12	13	3	61	76	*R** *r*
Totals . . .	*UV*	*66*	*31*	*25*	*7*	*129*	*152*	0·30 0·8
24	V	(52 *R*)		(24 *r*)		70	76	...
12	V	32	14	18	7	71	76	...
8	V	35	14	19	7	75	76	...
Totals . . .	*V*	*67*	*28*	*37*	*14*	*146*	*152*	6·21† 1
Grand Totals . .	*UV+V*	*133*	*59*	*62*	*21*	*275*	*304*	4·79† 0
			Sn	*sn*				
M×G (homozygous for *le, r*)								
24	UV	...	unclassifiable		...	72	72	...
12	UV	...	54	17	...	71	72	...
8	UV	...	54	14	...	68	72	...
Totals . . .	*UV*	...	*108*	*31*	...	*139*	*144*	0·54
24	V	...	unclassifiable		...	70	72	...
12	V	...	48	18	...	66	72	...
8	V	...	52	15	...	67	72	...
Totals . . .	*V*	...	*100*	*33*	...	*133*	*144*	0·00
Grand Totals . .	*UV+V*	...	*208*	*64*	...	*272*	*288*	0·31

* Seeds were selected for *R* : *r* before planting. χ^2 refer to *Sn* segregation within *R* plants and *r* pl

† Significant at $P < 0.05$.

continuous light and with unvernalised seed a continuous bimodal distribution is obtained which, with a small chance of misclassification

(about 5 per cent.), can be interpreted as a segregation of three lates : one early. Prior vernalisation and continuous light gives an almost unimodal segregation. Under short days, whether the progeny is vernalised or not, a discontinuous segregation of three late : one early is obtained. Change of photoperiod from P_8 to P_{12} and prior vernalisation simply increases or decreases the size of the discontinuity. These treatments do not break up the late segregates into definite groups some reacting to vernalisation but not to photoperiod and *vice versa*. The main genetic difference between Massey and Telephone thus resides in a major dominant gene which apparently causes the late-flowering habit by the induction of vernalisation and photoperiodic responses. Following Wellensiek (1925) I propose to designate this locus with the symbol *Sn*.

TABLE 4

Analysis of χ^2 *for* Sn/sn *segregation*

Source of χ^2	M×T		M×G		A×T			
					uncorrected		corrected	
	d.f.	χ^2	d.f.	χ^2	d.f.	χ^2	d.f.	χ^2
Genetic deviation .	1	0·02	1	0·31	1	3·94*	...	...
V treatment .	1	1·09	1	0·23	1	3·74*	1	3·41
P treatment .	2	0·41	1	0·60	1	0·07	1	0·07
P and V treatment	4	1·44	3	0·86	3	3·88	3	3·45

Table 4 gives analysis of χ^2 for the crosses M×T, M×G and A×T. χ^2 significant at 0·05 level shown by *. In the cross A×T there is a significant overall excess of *sn* plants with an apparent effect of V treatment. However, after correcting for the excess *sn* plants ($P = 0{\cdot}302$) the effect of V treatment becomes non-significant at the 0·05 level ($P = 0{\cdot}08$).

A χ^2 analysis is given in table 4. It shows that segregation at the *Sn* locus is independent of segregation at the *Le* locus. There is a slight but not significant deficiency of double recessives, *le*, *sn* ($\chi^2 = 6{\cdot}61$, $P = 0{\cdot}10$). The analysis of χ^2 can be followed further and used as a test for heterogeneity in segregation under five of the six different treatments. For the *Sn* locus this analysis is given in table 4. There are no significant χ^2 either for genetic deviation or for treatments or their interaction.

The results for the F_2 of Massey ×Greenfeast and Alaska ×Telephone are given in table 3. No F_1 seed was available for growth under constant environments. Under continuous light whether vernalised or not, accurate classification in late and early segregates is impossible. In the case of Greenfeast ×Massey the only major locus segregating is *Sn*. Tables 3 and 4 show that segregation at this locus agrees in all respects with the segregation in Massey ×Telephone F_2. There is again no suggestion that vernalisation or change of photoperiod splits the late segregations up into groups differing in response to these

environmental changes. Similarly the χ^2 analysis gives no significant effect on the segregations of either vernalisation or photoperiod.

In the cross Alaska ×Telephone, the two loci, *R* (round : wrinkled seed) and *Sn*, were segregating. There was a good 3 : 1 segregation at the *R* locus in F_2 and segregation at this locus is independent of segregation at the *Sn* locus although after vernalisation there seems to be a significant excess of *sn* plants in the *R* class. Unlike the other two F_2, there is an overall deficiency of late segregates in this F_2 ($\chi^2 = 3{\cdot}94$, $P < 0{\cdot}05$). An analysis of χ^2 for heterogeneity shows that the deficiency is associated with vernalisation ($\chi^2 = 3{\cdot}74$, $P < 0{\cdot}05$). If the analysis of χ^2 for heterogeneity is carried out correcting for the overall deficiency of lates, the heterogeneity due to vernalisation is just non-significant at the 5 per cent. level ($\chi^2 = 3{\cdot}41$, $P = 0{\cdot}08$). Further work is required using F_3 progeny to decide whether the deficiency of lates after vernalisation is caused by the appearance of late segregates in F_2 which show a greater response to vernalisation than the late parent.

Table 5 gives estimates of means and variances of the distributions of flowering node in parents F_1 and F_2. The variability of the early parents is unaffected by change of environment whilst for the late parent variability is greatly reduced by vernalisation and by long days. Similarly the variances of the early segregates in F_2 are relatively unaffected by environmental change whilst the late segregates behave very similarly to the late parent. It is noteworthy that the estimates of variance in the F_1 of Massey ×Telephone, although based on only five plants per sample, are always less than the corresponding variance of the late parent. Variability in F_1 is probably only slightly greater than in the early parent. Since the F_1 is phenotypically late in all other respects, the reduction in variability as compared with the late parent is to be interpreted as an example of genetic developmental homeostasis (Lerner, 1954), the homeostasis showing itself not as a reduction in total response to environmental variables but in a more *regular* response in the heterozygote than in the homozygote. As will be seen later the *Sn* gene has pleiotropic effects on node of formation of first leaf with four leaflets and also on stem length. There is no evidence of homeostasis with regard to these effects.

A comparison of the variances of the early and late segregates with the parental variances shows that in addition to segregation at the *Sn* locus, modifiers or polygenes or both must also be segregating. Let us compare the means and variances of the early segregates with those of the early parent (table 5). In all cases the early segregates flower at a higher node than the early parent. The difference is about 2-3 nodes. The variances of the distribution of flowering node of the segregates are over ten times larger than the variances of early parent with the distributions of the segregates regularly transgressing the upper but not the lower parental limits. It is also clear that neither vernalisation nor change in photoperiod from P_8 to P_{12} alters the mean

flowering node or the variance of the early segregates. These facts show that several polygenic loci are segregating in F_2. The polygenes cannot be considered as modifiers of the physiological system on which the *Sn* locus works. They presumably work through some physiological system of flower-control other than the system controlling the development of responses to vernalisation and photoperiod.

TABLE 5

Mean and variance of flowering node, F, in parents F_1 and F_2

	Vernalisation	Variety or Segregate	Photoperiod					
			P_{24}		P_{12}		P_8	
			$\bar{x}$	Var.	$\bar{x}$	Var.	$\bar{x}$	Var.
sn EARLY VARIETY OR SEGREGATE	UV	Massey	9·10	0·10	9·10	0·10	9·10	0·10
		F_2 segregate M×T	11·50	0·74	11·26	1·29	11·04	2·13
		Alaska	10·00	0·00	10·20	0·18	10·20	0·18
		F_2 segregate A×T	...	...	13·25	2·86	12·38	2·78
		F_2 segregate M×G	...	...	11·18	1·28	11·50	1·18
	V	Massey	9·60	0·27	9·80	0·18	10·00	0·00
		F_2 segregate M×T	...	...	11·83	1·06	11·26	0·86
		Alaska	10·30	0·23	10·20	0·18	10·10	0·10
		F_2 segregate A×T	...	...	12·04	1·04	12·15	0·70
		F_2 segregate M×G	...	...	11·39	1·31	12·20	2·16
Sn LATE VARIETY OR SEGREGATE	UV	Telephone	18·00	0·74	23·95	2·06	29·00	7·68
		F_1 M×T	17·60	0·30	24·00	0·50	26·00	0·00
		F_2 segregate M×T	16·56	1·56	23·19	10·50	26·99	21·48
		F_2 segregate A×T	(15·88)	(6·95)	21·88	3·36	23·84	11·43
		Greenfeast	16·50	0·50	25·70	2·68	29·43	3·62
		F_2 segregate M×G	(15·70)	(7·58)	25·71	16·84	27·81	17·06
	V	Telephone	15·80	0·27	20·10	0·94	21·35	1·40
		F_1 M×T	15·00	0·00	19·80	0·70	20·80	0·70
		F_2 segregate M×T	(13·29)	(3·73)	19·47	3·22	21·17	7·43
		F_2 segregate A×T	(14·07)	(3·21)	18·63	2·15	20·16	4·01
		Greenfeast	14·90	0·32	19·80	0·92	22·00	2·00
		F_2 segregate M×G	(13·47)	(3·58)	21·60	5·07	23·27	9·04

Figures in brackets give mean and variance of total F_2 distribution where progeny is not classifiable into *Sn* and *sn*.

If we consider the late segregates, it is clear that the means of the distributions of flowering node are affected by photoperiod and vernalisation in the same way as the means of the late parents. The distributions as a whole respond to vernalisation and change of photoperiod in the same way and to approximately the same extent as the late parent. The variances of the distributions of the late segregates are greater than the variances of the late parents, the F_2 distributions tending to transgress both upper and lower parental limits. The simplest interpretation of these facts is that we are dealing with segregation of a system of genes modifying the action of the *Sn* gene. Whether

there is also a system of polygenes, segregation of which is capable of changing the node of first flower independently of the action of the *Sn* gene, is at present impossible to demonstrate directly. However, by comparison with the distributions of the early segregates it is probable that a polygenic system independent of the system of modifiers does exist. Further tests in F_3 are required to identify and separate genetically the two systems.

These simple experiments thus afford proof that the position of first flower in these varieties depends on at least three identifiable systems of genes. Without the design of experimental tests based on detailed knowledge of the pattern of behaviour of the parental varieties, this analysis could not have been made. The analysis of parental behaviour enabled the environment which maximises the parental differences to be selected for testing the genetic segregations. This precaution seems an essential one for experiments of this kind. Thus, Davern *et al.* (1957) have attempted to analyse genetically the differences in flowering behaviour of varieties of subterranean clover without taking this precaution. They state : " By manipulation of the sowing date one should be able to obtain discrete segregations into flowering and non-flowering types superficially suggesting major gene inheritance." It is not quite clear what the word " superficially " means in this connection. They then discuss the conflicting studies on the inheritance of spring and winter habit in cereals and suggest that " This confusion could be largely explained by the theory that a continuous variable, the genetic control of cold requirement for competence to flower, underlies the two phenotype classes, spring and winter habit." There seems little doubt that, in the diploid cereal rye, segregation at a single locus of a pair of alleles determining a major requirement for vernalisation, does occur (Purvis, 1939). We could hardly expect to demonstrate the existence of this major gene by sowing an F_2 in the autumn. Spring sowings in long days are necessary to allow the effect of the major gene-difference to appear. In the hexaploid cereals, the genetic situation will undoubtedly be more complex ; but experiments planned (*a*) to reduce environmental fluctuations to a minimum and (*b*) to allow the difference in pattern of behaviour best to express itself, are necessary before concluding that a continuous genetic variable for cold requirement underlies the difference between spring and winter flowering habit.

This problem is, perhaps, not so academic as might at first sight appear. A strain of subterranean clover corresponding to the early pea varieties would be a strain with some considerable agronomic potentiality. The only way to select it from a segregating F_2 would be to grow the F_2 under conditions which would prevent (or delay) the flowering of all segregates with a requirement for vernalisation or long days. Aitken's work shows the type of environment required. Study of F_2 segregation at high temperatures and in short days will probably bring to light variation in subterranean clover homologous

genetically and physiologically with that present in these varieties of peas.

5. PLEIOTROPIC EFFECTS OF THE *Sn* GENE

We have seen already that the dominant *Sn* gene has three pleiotropic effects on flowering—a delaying action and the induction of competence to respond to vernalisation and photoperiod. The data available also allow some consideration of the pleiotropic effect of this gene on growth in length of stem and on leaf pattern. The Massey × Telephone F_2 allows us to test the action of this gene on both an *Le*

TABLE 6

Pleiotropic effects on vegetative characteristics of segregation at Sn *locus*

Cross	Genetical background	C					L				
		UV			V		UV			V	
		P_{24}	P_{12}	P_8	P_{12}	P_8	P_{24}	P_{12}	P_8	P_{12}	P_8
M×T .	*Le, Sn*	13·63	14·78	15·52	14·38	15·41	25·49	19·69	19·01	17·12	14·24
	Le, sn	11·38	11·04	10·50	11·27	10·95	27·83	25·59	26·12	19·68	18·00
	le, Sn	13·30	13·16	15·42	13·79	15·83	8·44*	7·20*	6·79	6·32*	4·58*
	le, sn	10·60	10·57	10·50	11·25	9·67	8·80	8·07	10·13	7·25	5·67
A×T .	*Le, Sn*	...	13·43	13·73	11·70	12·29	...	22·16	17·16	24·26	18·51
	Le, sn	...	10·71	10·88	10·24	10·58	...	25·76	24·16	28·28	21·19
M×G .	*le, Sn*	...	15·50	15·05	13·23	...	...	7·09	6·08	5·27*	4·99
	le, sn	...	10·41	10·66	10·67	10·85	...	8·41	8·50	5·78	6·03

Table 6 gives mean values of C (node of first four-leaflet leaf) and L (length in cm. of internodes 5-9) in F_2 progenies segregating for *Sn*/*sn* grown under six different environments.

All differences ($C_{Sn}-C_{sn}$) on both *Le* and *le* background are significant at $P<0{\cdot}001$ (*t* test).

All differences ($L_{Sn}-L_{sn}$) on *Le* background are significant at $P<0{\cdot}01$; on *le* background, unstarred differences are significant at $P<0{\cdot}02$, starred differences * are not significant at $P=0{\cdot}05$ in most cases owing to small numbers of *le, sn* plants available for comparison. In all cases $L_{Sn}<L_{sn}$.

and an *le* background. Similar comparisons are available from the Alaska ×Telephone cross (*Le*) and the Massey ×Greenfeast (*le*).

Table 6 gives the mean values for C and L in the three F_2 grown in those environments where classification with regard to *Sn*/*sn* is possible. There is no doubt that *Sn* raises the node of formation of the first leaf with four leaflets in all F_2 grown under all environments. The size of the rise varies between crosses and in the different environments. In Alaska ×Telephone, substitution of *Sn* for *sn* gives a rise of under 3 nodes in unvernalised plants in short days. In the other crosses the rise is about 5 nodes. As would be expected from the behaviour of the pure varieties, photoperiod and vernalisation have no effect in *sn* plants. In *Sn* plants, however, there is a small response to photoperiod and to vernalisation, the responses, as in the pure varieties, being smaller than those of the flowering node. Similar responses occur in both *Le* and *le* plants.

As regards L, the recessive *sn* plants in all comparisons available are taller than *Sn*. The effect is apparently present on both *Le* and *le* backgrounds although owing to the small numbers of double recessive plants available in the Massey × Telephone cross, only one of the differences is significant at the 0·05 level in the individual comparisons available in this F_2. As in the pure varieties, L decreases with decrease in photoperiod and with prior vernalisation except in the case of Alaska × Telephone where the vernalisation response seems to be anomalous (*cf.* behaviour of Alaska).

Summing up, the dominant *Sn* allele in comparison with the *sn* allele has the following five functions : (*a*) it delays flowering to a higher node ; it induces competence to respond to (*b*) photoperiod and (*c*) vernalisation ; (*d*) it delays the appearance of first leaf with

TABLE 7

Effect of segregation at Le *locus on node of first flower*

Genetic background	UV			V	
	P_{24}	P_{12}	P_8	P_{12}	P_8
Le Sn	16·41	23·42	27·81	19·63	21·29
Le sn	11·48	11·07	11·44	11·89	11·40
le Sn	16·82	21·87	23·83	19·00	20·69
le sn	11·60	12·00	11·00	11·50	10·33

Table 7 gives mean node of first flower, F, in F_2 of Massey × Telephone grown under different environments.

four leaflets and (*e*) it reduces growth in length of stem. Although the genetic tests for these five pleiotropic functions are not complete, there is at present no evidence that the apparent pleiotropy is due to tight linkage of several loci each with a single function.

6. MODIFYING EFFECTS OF THE *Sn* GENE OF SEGREGATION AT OTHER LOCI

We have seen that there is evidence of (1) a system of polygenes controlling node of first flower by a mechanism other than that controlled by the *Sn* gene and (2) a system of genes modifying the effect of *Sn* and presumably ineffective in the absence of *Sn*. The crosses allow us to test the effects of segregation at two other major loci on *Sn*/*sn*. They are the *Le* and *R* loci. Rasmussen (1935) has claimed that the *le* gene is dominant for a small delay in time to flower.

R, segregating in Alaska × Telephone, has no effect detectable in the experiments reported here on flowering node.

Table 7 shows that in the Massey × Telephone cross there is some slight evidence that *Le* on a background of *Sn* may slightly raise the node of first flower. In four out of the five comparisons possible, *Le*

plants flower later than *le*. The difference only reaches statistical significance in one case (P_{12}, UV, $P<0{\cdot}01$). On an *sn* background segregation at the *le* locus has little if any effect.

The comparisons in this cross suffer because of the great increase in variance on flowering node in the F_2 segregates. Two F_2 progenies from Telephone ×Greenfeast and Alaska ×Massey grown in the winter of 1954 at Hobart are available for a more precise comparison. In a progeny of 42 plants from T ×G, the mean flowering node for *Le* (31 plants) was 22·84 ; for their 11 *le* sisters, the mean flowering node was 22·18. The difference is just significant at the 0·05 level. For the A ×T cross corresponding figures are *Le* (34 plants) mean flowering node = 10·03 ; *le* (14 plants) mean flowering node = 9·93 ; the difference is not significant.

It is thus possible that the genes at the *Le* locus in addition to their major effect of stem length, are modifiers of the action of the *Sn* gene, having no effect on the *sn* allele. Further tests are being carried out to clarify this point.

7. PHYSIOLOGICAL GENETICS OF FLOWERING

It remains to attempt to correlate all these observations into a coherent scheme. Let us start our physiology from the genetical facts. The *Sn* gene is dominant for later flowering and it is dominant for competence to respond to photoperiod and vernalisation. The easiest way of looking at these facts is to suggest that the *Sn* gene provides the substrate for the photoperiodic and vernalisation reactions. If this substrate is a colysanthin, all these effects of the *Sn* gene are simply explained. Vernalisation and long days destroy colysanthin and so flowering is made earlier after cold treatment or growth in long days. This scheme enables us to predict that the vernalisation and photoperiodic responses should be competitive (as has been found) and not complementary as would be expected on the older ideas of a chain of reactions, leading to the synthesis of a positive flower hormone with an early link in the chain temperature-sensitive and a later link light-sensitive.

This simple picture enables us to explain the results of grafting and leaching experiments. The grafting experiments are of three types : (*a*) *sn*-*Sn* grafts as reported in Paton and Barber (1955) and interpreted as a transfer of a colysanthin from a late stock to an early scion ; (*b*) the demonstration that the amount of colysanthin in a vernalised *Sn* stock is less than in an unvernalised stock by use of *sn* scions for bio-assay ; (*c*) the induction of a small photoperiodic response in *sn* scions grafted into leafy *Sn* stocks, the composite plants being grown in long (no delay in flowering *sn* scion) and short days (1-2 node delay in flowering) (Barber and Paton, unpublished). Similarly as shown by Sprent and Barber (1957) the earlier flowering of young *Sn* cuttings soaked in water is easily explained by the leaching out of colysanthin (or possibly its destruction during metabolism under conditions of

semi-starvation). Scions of early varieties are unchanged by the leaching.

It has so far not been possible to isolate and chemically characterise this gene-product, a colysanthin. However, recent experiments have greatly improved the possibilities of efficient bio-assay. Barber *et al.* (1958) have shown that 1 μg. of gibberellic acid in alcoholic solution applied to the dry testa of intact seeds of the early variety Massey will cause a delay in flowering of 10-20 per cent. in terms of node of first flower. The delay is unaffected by vernalisation and photoperiod, so that although gibberellic acid is a colysanthin it cannot be chemically identical with the colysanthin produced by the *Sn* gene. However, the existence of a pleiotropic effect on stem length at the *Sn* locus, may mean that colysanthin is metabolically related to gibberellic acid in some way. Haupt (1957), using the technique of aseptic culture of embryos without cotyledons, has shown that yeast extract contains a substance capable of inducing a photoperiodic response in embryos of an early variety. Under short days the treated embryos flower at a later node than embryos grown in total darkness or in long days. Haupt attempts to explain his results on the basis of a " Blühhormon ". They seem more easily explicable (as do the similar results of Tashima (1953) on radish) on the basis that yeast extract contains a substance which in short days acts as a precursor of a colysanthin. Further experiments with diffusates, macerates of peas, yeast, etc., and leach-waters are continuing.

There remain two further problems. The first is the question of whether flower-stimulating substances are also present in peas. This problem may be connected with the existence of a polygenic system controlling flowering by means other than the *Sn* system of inhibitors. The second problem is the wider question of the amount of variation in the genetics and physiology of control of flowering which we may expect in different species of flowering plants.

As regards the question of a " florigen ", Haupt (1952) and Cruickshank (unpublished) have shown that pea embryos of early varieties, when grown without cotyledons on a medium unsupplemented with yeast extract, may flower at a higher node than intact plants. Haupt has concluded that this is evidence for the presence of a florigen. However, growth is very slow and poor and the effect may be a secondary one. There is, also, the more general argument for the presence of stimulators in the widely-held doctrine of organ-forming substances deriving from Sachs' speculations. An early variety of pea produces 5 to 7 vegetative nodes whilst growing in the pod. On germination it produces a further 3 to 5 vegetative nodes and then suddenly initiates flowers at the 8-10th node. It is still an attractive speculation to explain this pattern of development by postulating that a florigen is formed soon after germination.

We have seen that polygenes can cause a considerable variation in node of first flower in early non-photoperiodic varieties and segregates.

Extremes vary from node 6 (in an Acacia stock) to at least node 13 or 14. Is this variation controlled by the timing of florigen synthesis? There is scarcely any direct evidence either way. But the genetics and physiology can easily be linked up in a scheme such as is given in fig. 6 where florigen is shown as a precursor of colysanthin. It may be that vernalisation and long-day treatment act by reversing the action of the *Sn* gene. The possibilities are too numerous to specify in detail. However, further combined genetic and physiological work will undoubtedly limit the possibilities. If the relationship is as shown, with polygenes largely concerned with the synthesis and use of precursors of florigen and florigen itself a precursor of colysanthin, we can predict that the effect of the *Sn* gene will vary according to the polygenic background. With the scheme shown we would expect the photoperiod and vernalisation responses of *Sn* to be smaller when the

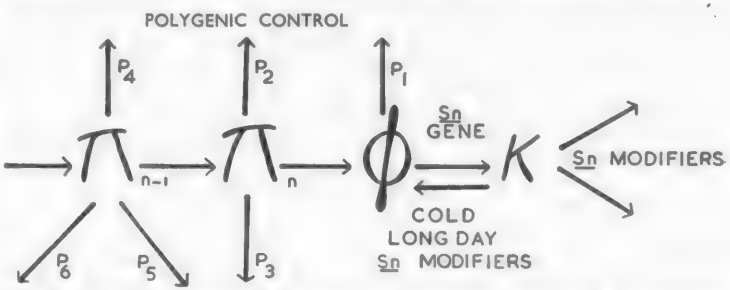


FIG. 6.—Scheme showing one of the many possible relationships of genes, gene-products and environment in the control of flowering in peas. π_n are precursors of florigen, ϕ, which is assumed to be a precursor of colysanthin, κ. Long days and vernalisation are assumed to reverse the action of *Sn*. The polygenic system, P_n, is assumed to regulate the supply of π_n by controlling utilisation of π_n in other metabolic processes. The modifiers of *Sn* including the *Le* locus may act by utilising some of κ or by altering the mechanism of responses to vernalisation and photoperiod.

gene is introduced into an *sn* plant flowering at a high node than when it is introduced into an *sn* flowering at a low node. It is hoped to select such stocks from F_3 progenies of early × late.

As regards the physiological fate of colysanthin during its destruction by vernalisation and by long days, it may be possible to isolate genes controlling these reactions. This would help greatly in understanding the physiology.

As regards the second problem of the variation in the genetical and physiological control of flowering within the Angiosperms, it is a natural first hypothesis to assume a single system of control (*cf.* Lang, 1952). However, the evidence is becoming overwhelming that fundamental differences exist between different species. As we have seen there is little doubt that the system in peas is based on a colysanthin; in other species of plant the evidence is almost as strong for a florigen base. Recent work has shown that the same substance (gibberellic acid) can act as a florigen (or vernalin) in plants with a

vernalisation requirement and in plants with an obligate or facultative long-day requirement. It can act as a colysanthin in short-day plants and also in facultative long-day plants. There is, thus, an almost complete variation in competence to respond to the one substance within four or five species of flowering plant.

How many other substances exist which have been used for the control of flowering in the genetically different mechanisms of different species is unknown. But it is unlikely that all the different mechanisms rely on a single substance. The control of time of flowering is a control of prime adaptive value. It is a control which must be sufficiently flexible to cope with the irregularities of seasons as well as with secular changes in climate. Evolutionary changes in control to meet the secular changes have been brought about by natural selection working on adaptively random gene-mutation and recombination. The evolutionary changes in gene-frequency must be expected to control both the chemical type of evocator and the competence (whether inhibition or stimulation) to respond to a specific evocator. As in so many adaptive responses we must expect the same end-result—the adaptive control of flowering—to have been arrived at in several genetically and chemically different ways (Barber, 1956).

8. SUMMARY

1. Early varieties of *Pisum sativum* are non-photoperiodic and non-vernalisable (or slightly negatively vernalisable). Late varieties are quantitative long-day plants with a positive reaction to vernalisation. The reactions to photoperiod and vernalisation compete with one another. Prior vernalisation reduces the photoperiodic response.

2. The interaction of genetic segregation and environment shows that the late varieties differ from the early by possessing a dominant, *Sn*, gene which causes later flowering by inducing a response to photoperiod and vernalisation. It is suggested that the *Sn* gene causes these effects by producing a flower-delaying substance (or *colysanthin*). This gene-product must be destroyed before flowering can take place. Colysanthin is preferentially destroyed at low temperatures and in long days.

3. The *Sn* gene has several other pleiotropic effects. It raises the node at which the first leaf with four leaflets is produced. It also slightly reduces growth in length of the stem, the reduction being about 10 per cent. of the reduction brought about by genes at the *Le* locus.

4. In addition to the major control by the *Sn* locus, there are two other gene systems controlling flowering. The first is a system of genes modifying the action of the *Sn* gene. The second is a system of polygenes which alter the node of first flower by a physiological mechanism other than by way of colysanthin.

5. Segregation at the *R* locus has no effect on flowering. The *Le* locus may be a modifier of *Sn*, the *Le* allele causing a slight delay in flowering when associated with the *Sn* gene.

6. There is some evidence that heterozygous *Sn*, *sn* plants show a more regular homeostatic response to environmental changes than the *Sn* parent.

Acknowledgments.—It is a pleasure to express my indebtedness to Drs G. W. Beadle, J. Bonner and F. W. Went for so kindly allowing me to work in the laboratories of the Division of Biology, California Institute of Technology during the tenure of a Special Fellowship of the Rockefeller Foundation. The work in Hobart has been made possible only by the generosity of the Rural Credits Division of the Commonwealth Bank of Australia and the Research Grants Committee of the University of Tasmania. Mr R. H. Cruickshank and Dr D. M. Paton were responsible for the genetic programme in Hobart.

9. REFERENCES

AITKEN, Y. A. 1955. Flower initiation in pasture legumes I and II. *Austral. J. Agric. Res.*, *6*, 212.

BARBER, H. N. 1956. The natural history of natural selection. Presidential Address Section M., A.N.Z.A.A.S. *Austral. J. Sci.*, May, 148.

BARBER, H. N., AND PATON, D. M. 1952. A gene-controlled flowering inhibitor in Pisum. *Nature*, *169*, 592.

BARBER, H. N., MURFET, I. C., AND SPRENT, J. 1958. *Nature*, *182*, 1321.

BONNER, D. M., HAAGEN-SMIT, A. F., AND WENT, F. W. 1939. Leaf growth hormones I. *Bot. Gaz.*, *101*, 128.

BONNER, J., AND THURLOW, J. 1949. Inhibition of photoperiodic induction by applied auxin. *Bot. Gaz.*, *110*, 613.

BONNER, J., AND GALSTON, A. W. 1952. *Principles of Plant Physiology*. Freeman: San Francisco.

BONNER, J., AND HAMNER, K. C. 1938. Photoperiodism in relation to hormones as factors in floral initiation and development. *Bot. Gaz.*, *100*, 388.

BRIAN, P. W., AND HEMMING, H. C. 1955. The effect of gibberellic acid on shoot growth of pea seedlings. *Physiol. Plant*, *8*, 669.

BUNSOW, R., AND HARDER, R. 1956. Blütenbildung von *Bryophyllum* durch Gibberellin. *Naturwiss*, *43*, 479.

CLARK, H. K., AND KERNS, K. R. 1942. Control of flowering with phytohormones. *Science*, *95*, 536.

DAVERN, C. I., PEAK, J. W., AND MORLEY, F. H. W. 1957. The inheritance of flowering time in *Trifolium subterraneum*. *Austral. J. Agric. Res.*, *8*, 121.

DENFFER, D. VON. 1950. Blühhormon oder Blühhemmung? *Naturwiss*, *37*, 296 and 317.

FINCH, L. R., AND CARR, D. J. 1956. Nucleic acid content of petkus rye embryos in relation to vernalisation and devernalisation. *Austral. J. Biol. Sci.*, *9*, 355.

GREGORY, F. G. 1948. The control of flowering in plants, in *Growth, Symp. Soc. Expt. Biol.*, *2*, 75.

GUTTRIDGE, C. G. 1956. Photoperiodic promotion of vegetative growth in the cultivated strawberry plant. *Nature*, *178*, 50.

HAMNER, K. C. 1948. Factors governing the induction and development of reproductive structures in plants, in *Growth, Symp. Soc. Expt. Biol.*, *2*, 104.

HARDER, R. 1948. Vegetative and reproductive development of *Kalanchoe blossfeldiana* as influenced by photoperiodism in *Symp. Soc. Expt. Biol.*, *2*, 117.

HARDER, R., AND BUNSOW, R. 1957. Zusammenwirken von Gibberellin mit photoperiodisch. . . . Vorgängen bei Kalanchöe. *Naturwiss*, *44*, 454.

HAUPT, W. 1952. Untersuchungen über den Determinationsvorgang der Blütenbildung bei *Pisum sativum*. *Z. Bot.*, *40*, 1.

HAUPT, W. 1957. Photoperiodische Reaktion bei einer als tagneutral geltenden Sorte von *Pisum sativum*. *Ber. Bot. Gessel*, *70*, 191.

HIGHKIN, H. R. 1955. Flower-promoting activity of pea seed diffusates. *Plant Physiol.*, *30*, 390.

HIGHKIN, H. R. 1956. Vernalisation in peas. *Plant Physiol.*, *31*, 399.

HOWELL, M. J., AND WITTWER, S. H. 1954. Chemical induction of flowering in the sweet potato. *Science*, *120*, 717.

LANG, A. 1948. Genetic der Vernalisation und des Photoperiodismus in *Vernalisation and Photoperiodism*, ed. Murneek, A. E., and Whyte, R. O. *Chron. Bot.*, Waltham, Mass.

LANG, A. 1952. Physiology of flowering. *Ann. Rev. Pl. Phys.*, *3*, 265.

LANG, A. 1956. Induction of flower formation in biennial *Hyoscyamus* by treatment with Gibberellin. *Naturwiss*, *43*, 284.

LANG, A. 1956. Gibberellin and flower formation. *Naturwiss*, *43*, 544.

LANG, A., AND MELCHERS, G. 1943. Die photoperiodische Reaktion von *Hyoscyamus niger*. *Planta*, *33*, 653.

LANGRIDGE, J. 1957. Effect of daylength and Gibberellic acid on the flowering of *Arabidopsis*. *Nature*, *180*, 36.

LERNER, I. M. 1954. *Genetic Homeostasis*. Oliver and Boyd, Edinburgh.

LIVERMAN, J. L., AND LANG, A. 1956. Induction of flowering in long day plants by applied indoleacetic acid. *Plant Physiol.*, *31*, 147.

LEOPOLD, A. C., AND GUERNSEY, F. S. 1954. Flower initiation in the Alaska pea II. *Amer. J. Bot.*, *41*, 181.

LOCKHART, J. A., AND HAMNER, K. G. 1954. Effect of darkness and I.A.A. following exposure to SD on the floral response of *Xanthium*. *Bot. Gaz.*, *89*, 133.

MELCHERS, G., AND LANG, A. 1942. Auslösung von Blütenbildung bei Langstagspflanze *Hyoscyamus* in Kurztagesbedingungen mit Zucker. *Naturwis.*, *31*, 249.

MELCHERS, G., AND LANG, A. 1948. Die Physiologie der Blütenbildung. *Biol. Zbl.*, *67*, 105.

OVERBEEK, J. VAN. 1946. Control of flower formation and fruit size in the pine-apple. *Bot. Gaz.*, *108*, 64.

OWEN, F. V., CARSNER, E., AND STOUT, M. 1940. Photothermal induction of flowering in sugar beets. *J. Agric. Res.*, *61*, 101.

PATON, D. M., AND BARBER, H. N. 1955. Physiological genetics of *Pisum* I. *Austral. J. Biol. Sci.*, *8*, 231.

PELLEW, C. 1940. Genetical studies on the first reciprocal translocation found in *Pisum sativum*. *J. Genet.*, *39*, 363.

PURVIS, O. N. 1939. Studies in vernalisation of cereals V. *Ann. Bot.*, N.S., *3*, 719.

RASMUSSEN, J. 1935. Studies on the inheritance of quantitative characters in *Pisum* I. *Hereditas*, *20*, 161.

SCHWABE, W. W. 1956. Evidence for a flowering inhibitor produced in long days in *Kalanchoe blossfeldiana*. *Ann. Bot.*, N.S., *20*, (77), 1.

SHIGEURA, G. 1948. Blossum-bud formation and fruit setting in the litchi. *Univer. Hawaii Agric. Expt. St.*, 1946-1948, 138.

SKOK, J., AND SCULLY, N. J. 1954. Characteristics and movement of the flowering stimulus from the induced leaf of *Xanthium*. *Bot. Gaz.*, *116*, 142.

SPRENT, J. I., AND BARBER, H. N. 1957. Leaching of a flower inhibitor from late varieties of peas. *Nature*, *180*, 200.

TASHIMA, Y. 1953. Flower initiation in total darkness in a long-day plant *Raphanus sativus*. *Proc. Jap. Acad.*, *29*, 271.

TEDIN, H. O. 1923. Contributions to the genetics of *Pisum* III. *Hereditas*, *4*, 351.

THOMPSON, H. C. 1953. Vernalisation of growing plants in *Growth and Differentiation of Plants*. Ed. W. E. Loomis. Iowa State College, p. 178.

WELLENSIEK, S. J. 1925. Genetic monograph on *Pisum*. *Bibliog. Genetics*, *2*, 243.

WENT, F. W. 1957. *The Experimental Control of Plant Growth*. Chron. Bot., Waltham, Mass.

WHYTE, R. O. 1948. History of research in vernalisation in *Vernalisation and Photoperiodism*. Ed. Murneek, A. E., and Whyte, R. O. Chron. Bot., Waltham, Mass.

RESISTANCE IN MAIZE TO THE TROPICAL AMERICAN RUST FUNGUS, *PUCCINIA POLYSORA*

II. LINKAGE OF GENES Rpp_1 AND Rpp_2

H. H. STOREY and AUDRIE K. HOWLAND

East African Agriculture and Forestry Research Organisation

Received 5.v.58

In this paper we report studies of the genetic relationships of two genes in maize known to confer resistance to the tropical rust fungus, *Puccinia polysora* Underw. (Storey and Howland, 1957). The work was done at the East African Agriculture and Forestry Research Organisation at Muguga, Kenya. We owe much to Sir Joseph Hutchinson, F.R.S., for advice on interpretation and to Mr J. T. Walker for assistance in statistical estimations.

1. MATERIAL AND METHODS

(i) *Experimental methods*

Methods for inoculating young maize seedlings with uredospore suspensions remained unaltered (Storey and Howland, *loc. cit.*), except that in most experiments we inoculated the two rust races, EA.1 and EA.2, simultaneously to separate leaves of each seedling. Consequently phenotypes are defined in the form—

Reaction to EA.1/Reaction to EA.2.

Rust reactions were scored qualitatively as in our earlier work; but on the evidence there given they can be grouped in four classes :—

Class "0 1"—Previously "0 1, sub-class (*a*)"; characteristic reaction of R_1 * against EA.1.

Class "1"—Previously the range from "0 1 (*b*)" to "1++"; reaction of R_2 to EA.1 and EA.2.

Class "X"—Indeterminate class between "1" and "4", believed to indicate R_2 in combination with unfavourable minor genes.

Class "4"—Susceptible reaction of recessive genotypes; also of R_1 against EA.2.

(ii) *Sources of resistance genes*

Pure lines, derived from the three American introductions already studied (Storey and Howland, *loc. cit.*) and designated by their introduction numbers, were used.

AFRO.29.	Gene R_1.	Reaction "0 1/4".
AFRO.24.	Gene R_2.	Reaction "1/1".
AFRO.27.	Gene R_2 (?)	Reaction "1-X/1-X".

Two African lines, that had reacted "4/4" in many tests, provided double-recessive genotypes.

2. RESISTANCE GENES OF AFRO.29 AND AFRO.24

Five families, from crosses of AFRO.29 and AFRO.24, inoculated with Rust Race EA.1, all reacted "0 1" and were indistinguishable from plants carrying R_1 alone. Inoculated with EA.2, they reacted

* The gene designations Rpp_1 and Rpp_2 are shortened to R_1 and R_2 in this paper.

in the severe range of " 1 " and were indistinguishable from the heterozygote carrying $\mathbf{R}_2$ alone (table 1, series A).

Backcrossing the F_1 to the double recessive gave the eight families in table 1, series B, which segregated into the four phenotypes shown at the head of the table. Segregation evidently differed from that expected on independent assortment of genes. Analysis showed that segregation of $\mathbf{R}_1$, $\mathbf{r}_1$ and $\mathbf{R}_2$, $\mathbf{r}_2$, did not deviate significantly from 1 : 1 ratios ; but the χ^2 component for linkage was highly significant. The individual eight families (not recorded separately in the table) gave no evidence of heterogeneity.

TABLE 1

Crosses involving AFRO.29 ($\mathbf{R}_1$) and AFRO.24 ($\mathbf{R}_2$)

Series	Type of progeny	No. of families	Phenotypes and assumed genetic constitution			
			"01/1" $\mathbf{R}_1\mathbf{R}_2$	"01/4" $\mathbf{R}_1\mathbf{r}_2$	"1/1" $\mathbf{r}_1\mathbf{R}_2$	"4/4" $\mathbf{r}_1\mathbf{r}_2$
A . .	F_1	5	All*	0	0	0
B (1) . .	Backcross, $F_1 \times$ "4/4"	3	4	31	38	3
(2) . .	Backcross, "4/4" $\times F_1$	5	6	42	47	2
	Total .	8	10	73	85	5
C . .	F_2 (Repulsion)	11	207	74	95	1
D . .	F_2 (Coupling)	4	45	5	5	12

* In individual tests, 48 F_1 plants were all " 01 " against EA.1 ; and 96 were " 1 " against EA.2.

The reciprocal crosses did not differ significantly one from the other. Gametic segregation appeared therefore to be identical in each sex.

F_2 families were raised by selfing F_1 plants. Total numbers of the four phenotypes obtained in eleven families are recorded in table 1, series C. A single " 4/4 " plant appeared in a total of 377 ; this plant was further studied, (*a*) by reinoculation, when the " 4/4 " classification was again observed, and (*b*) by selfing, which gave an entirely " 4/4 " family (26 plants). This plant was therefore the double-recessive recombinant $(\mathbf{r}_1\mathbf{r}_2)(\mathbf{r}_1\mathbf{r}_2)$.

Of these F_2 totals, the $\mathbf{R}_1$, $\mathbf{r}_1$ segregation did not deviate significantly from a 3 : 1 ratio and the eleven families constituted a homogeneous group. In the $\mathbf{R}_2$, $\mathbf{r}_2$ segregation there was a just significant shortage of $\mathbf{r}_2$ genotypes (P lying between 0·02 and 0·05) ; and in this regard also the families were homogeneous, ten of the eleven contributing to the shortage of $\mathbf{r}_2$, although in only one of these did the individual departure reach significance. Despite this disturbance in one-factor segregation, there is still a large χ^2 for joint segregation, indicating linkage in the repulsion phase.

No explanation is evident for the deviation in the R_2, r_2 segregation in this generation. It is improbable that r_2 genotypes can have been classed " 1 " in error (although the converse can occur, as we show in the next section). There was no suggestion of lowered viability in any of the eleven families ; the ears were uniformly filled and failures in germination were few and appeared to be due to fungus infection. It is to be noted that in the backcrosses also there was a suggestion of shortage of r_2 genotypes, but not reaching significant level.

By selfing " 01/1 " phenotypes selected from the backcross families, we bred the four F_2 families recorded as series D in table 1. Individual segregations of the two gene pairs conformed very closely to 3 : 1 ratios and there was here no indication of any departure of R_2, r_2

TABLE 2

Selfs of " 01/1 " phenotypes selected from the coupling F_2 families, series D of table 1

Group	Probable genotype of parents	Numbers of families expected at 12·23% recombination	Observed numbers of families	Numbers of phenotypes observed			
				" 01/1 "	" 01/4 "	" 1/1 "	" 4/4 "
1	(R_1R_2) (R_1R_2)	5·01	3	56	0	0	0
2	(R_1R_2) (r_1r_2)	10·01	12	119	10	14	31
3	(R_1R_2) (R_1r_2)	1·39	0	...	...	...	...
4	(R_1R_2) (r_1R_2)	1·39	2	41	0	12	11
5	(R_1r_2) (r_1R_2)	0·19	1	7	2	3	0

from this ratio. The four families constituted a homogeneous group. The recorded segregation gave strong evidence of linkage in the coupling phase, which accords with the expected genotype (R_1R_2) (r_1r_2) of the parents.

Eighteen F_3 families were raised by selfing selected " 01/1 " phenotypes from the coupling F_2 of the previous paragraph. There are five possible double-dominant genotypes that could occur, each giving, on the assumption of linkage, a characteristic segregation in its selfed progeny. On this basis the eighteen families have been grouped as shown in table 2. The expected numbers of families in each group, on a recombination fraction of 12·23 per cent. (see below), appear in the third column of the table. The observed numbers appear not to deviate significantly from these expectations.

The twelve families in group 2 are believed to derive from (R_1R_2) (r_1r_2) and consequently provide further evidence on linkage in the coupling phase. The R_1, r_1 and R_2, r_2 segregations do not differ significantly from 3 : 1 and the twelve families are homogeneous ; the evidence for linkage is strong.

Three families in group 1 were homozygous double-dominant recombinants and provided the material that was the practical objective of the breeding.

On the assumption that gametic segregation is identical in the two sexes—which accords with the evidence of the backcrosses—values were estimated, by the method of maximum likelihood, of the recombination fraction for each of the sets of family totals, as follows :

Backcross (1)	9·21	per cent.
Backcross (2)	8·25	„ „
F_2 (repulsion)	12·23	„ „
F_2 (coupling—table 1)	16·13	„ „
F_2 (coupling—table 2)	14·98	„ „
Mean	12·16	„ „

A combined estimation of the recombination fraction from the whole of the data provided the figure of 12·23 per cent., with a standard error of $\pm$1·60 and confidence limits, at P = 0·05, of 9·10 and 15·37 per cent.

The observed numbers in each of the five sets of families showed no significant deviation from the expectations calculated on this recombination fraction.

3. THE RESISTANCE GENE OF AFRO.27

We have suggested (Storey and Howland, *loc. cit.*) that AFRO.27 carries $\mathbf{R}_2$, but modified by minor genes to give phenotypes of generally lower resistance than those carrying $\mathbf{R}_2$ from AFRO.24. If this is true, the cross with AFRO.29 should exhibit linkage similar to that of AFRO.24 by AFRO.29.

The F_1, inoculated with EA.1 reacted " 01 " ; and, with EA.2, reacted mostly " X " but with a scatter into grades of " 1 ". In addition, two plants out of 195 inoculated with EA.2 were classed " 4 ". One of these was selfed, and its progeny segregated, showing that the parent carried $\mathbf{R}_2$ and was not the double recessive suggested by its class " 4 " phenotype.

Eight backcross families were raised. In recording simultaneous tests against the two rust races, it was often difficult to decide between classes " X " and " 4 " for the EA.2 reaction. On the most favourable interpretation, by allocating all doubtful classifications to " X ", the segregation was as follows :—

	" 01, 1-X "	" 01, 4 "	" 1-X, 1-X "	" 4/4 "
Observed	4	56	27	20
Expectation on 12·23 per cent. recombination	6·54	46·96	46·96	6·54

The apparent $\mathbf{R}_1$, $\mathbf{r}_1$ segregation is not under suspicion, but that of $\mathbf{R}_2$, $\mathbf{r}_2$ deviates significantly from a 1 : 1 ratio, the " 4 " class being in large excess.

Five selected " 4/4 " phenotypes were selfed. Only one gave a uniform " 4/4 " progeny and was the double recessive. Four progenies segregated and their parents therefore carried $\mathbf{R}_2$ despite their " 4/4 " classification. There is therefore a further suggestion that minor genes from AFRO.27, and possibly from the susceptible parent of the backcross, may degrade an $\mathbf{R}_2$ genotype to full susceptibility. Evidently the discrepancy in the F_2 totals is mainly ascribable to misclassification of genotypes ; and if it be assumed that only one in five of the recorded " 4/4 " phenotypes is the double-recessive genotype, the remaining four being properly classed with " 1-X/1-X ", the observed totals do not diverge widely from the expectation based on 12·23 per cent. recombination.

The identity of the resistance gene in AFRO.27 remains uncertain, but there is no conclusive evidence that it is not $\mathbf{R}_2$.

4. SUMMARY

When pure lines carrying $\mathbf{Rpp}_1$ and $\mathbf{Rpp}_2$ (hereafter designated $\mathbf{R}_1$ and $\mathbf{R}_2$) were crossed, the progeny gave the characteristic reaction of the former if inoculated with the rust race EA.1 and that characteristic of $\mathbf{R}_2$ if inoculated with EA.2. Backcrosses and repulsion and coupling F_2's were classified by simultaneous inoculation of separate leaves of each seedling with the two rust races. Observed segregations showed that the two genes are linked, with a recombination fraction estimated at 12·23 per cent. and confidence limits ($P = 0{\cdot}05$) at 9·10 and 15·37 per cent.

From an F_3 generation families were selected that were the pure double-dominant recombinant $(\mathbf{R}_1\mathbf{R}_2)(\mathbf{R}_1\mathbf{R}_2)$.

The $\mathbf{R}_1$ line was also crossed with a second pure line believed to be carrying $\mathbf{R}_2$ but modified towards lowered resistance by minor genes. It was found that genotypes carrying this resistance gene might sometimes be modified to full susceptibility ; but, if account were taken of this, the evidence did not disprove the supposed identity of the gene.

5. REFERENCE

STOREY, H. H., and HOWLAND, A. K. 1957. Resistance in Maize to the tropical American rust fungus, *Puccinia polysora* Underw. I. Genes $\mathbf{Rpp}_1$ AND $\mathbf{Rpp}_2$. *Heredity, 11*, 289-301.

USE OF MITOTIC SEGREGATION FOR ASSIGNING GENES TO LINKAGE GROUPS IN *ASPERGILLUS NIDULANS*

E. FORBES
Department of Genetics, University of Glasgow

Received 22.v.58

1. INTRODUCTION

THE difficulties encountered in the early stages of mapping the chromosomes of some organisms can be reduced by using mitotic segregation and recombination. In *Aspergillus nidulans* mitotic segregation gives rise to two types of segregants ; diploids produced as a consequence of mitotic crossing over, and haploids produced as a consequence of a process as yet not well understood and which for short has been called haploidisation (Pontecorvo, Tarr-Gloor and Forbes, 1954 ; Pontecorvo and Käfer, 1956, 1958).

Haploid segregants arising from a multiply marked heterozygous diploid carry either one or the other member of each homologous chromosome pair of the parent diploid. As haploidisation does not involve crossing over, whole chromosomes segregate as units and recombine as units with other non-homologous chromosomes. On the other hand, diploid segregants arising from the same heterozygous diploid as a consequence of mitotic crossing over are usually homozygous for only one chromosome segment (distal to the position of crossing over) and still heterozygous for the rest of the other markers. The two types of segregants can be distinguished from one another by means of various criteria. The frequency of both types is low, the haploids usually in the minority.

A diploid heterozygous for one marker in each linkage group gives haploid segregants showing all combinations of these unlinked markers. On the other hand, two markers in the same linkage group but in the " repulsion " (" trans ") arrangement will not usually appear together among haploid segregants. This means that an unlocated mutant reveals its linkage group by not appearing together with the marker with which it is in repulsion. Thus the problem of identifying the linkage group of an unlocated mutant is that of selecting haploid segregants and classifying them for recombination or failure of recombination between the unlocated mutant and each of the tester markers.

Since haploid segregants are rare, methods facilitating selection are necessary. A possible way to do this is to take advantage of the fact that haploid segregants show free recombination between markers on different chromosome pairs and complete linkage between markers on the same chromosome pair. Thus, among haploid segregants showing a recessive or incompletely dominant marker one half show another unlinked recessive or incompletely dominant marker. In contrast to this, among diploid segregants only a fraction of one per

cent. of those homozygous for one recessive or incompletely dominant marker are homozygous for another recessive or incompletely dominant marker on a different chromosome pair or on a different arm of the same pair.

Thus, if we select simultaneously for two recessive or semi-dominant markers either on different chromosomes or on different arms of the same chromosome, we select mainly haploids. Among them the proportion of diploid segregants homozygous for both markers will be negligible.

The markers for this " combined selection " (Käfer, 1958, p. 114) should lend themselves to automatic screening. For example, a partially dominant marker *Acr1* in heterozygous condition *Acr1*/+ confers partial resistance to acriflavine. The homozygote *Acr1*/*Acr1* and the haploid *Acr1* are resistant to a higher concentration of acriflavine and can therefore be automatically selected on medium with high acriflavine concentration (Roper and Käfer, 1957).

As another example, a recessive suppressor *su1ad20* (which will be abbreviated to *su*) in homozygous or haploid condition allows normal growth of homozygotes *ad20*/*ad20* or haploid *ad20* on adenineless medium on which the parent diploid $\frac{su1ad20 \;\; ad20}{+ \;\; ad20}$ grows poorly (Pritchard, 1955).

As a third example, segregants *w* or *w*/*w* (with colourless, " white " conidia) from a diploid *w*/+ (coloured conidia) can be identified by inspection and isolated. So also can *y* or *y*/*y* segregants (yellow conidia) from a diploid *y*/+ (green). (*w* is epistatic to *y* and y^+.)

A combined selection, on adenineless medium with an adequate concentration of acriflavine, from a $\frac{su \;\; ad20}{+ \;\; ad20} \;\; \frac{Acr1}{+}$ heterozygous diploid, will pick out haploid segregants *su ad20 Acr1* and a few diploid segregants $\frac{su \;\; ad20}{su \;\; ad20} \;\; \frac{Acr1}{Acr1}$

Another combined selection, on adenineless medium, can screen *su ad20 w* (adenine independent, colourless) from $\frac{su \;\; ad20}{+ \;\; ad20} \;\; \frac{w}{+}$. The screening is automatic in respect to *su*, and visual in respect to *w*.

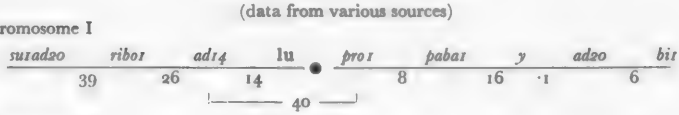
The technical requirements indicated above—automatic selection of haploids and one marker in each linkage group besides those required for selection—have been partially fulfilled by preparing two haploid tester strains. They were obtained by combining the required markers by appropriate crosses. A haploid strain carrying an unlocated mutant can be combined with either of these tester strains to form a heterozygous diploid. The two tester strains prepared so far do not have all eight linkage groups marked, but one (Tester Strain A) has linkage groups I, II, III, IV, V and VII marked, and the other (Tester Strain B) linkage groups I, II and VI. Any mutant, which fails to be

TABLE

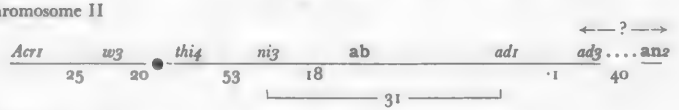
Mutants used in the present work

(data from various sources)

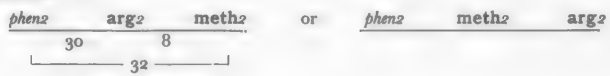
Chromosome I



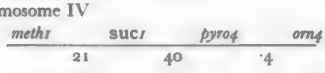
Chromosome II



Chromosome III



Chromosome IV



Chromosome V

lys5

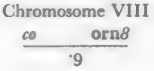
Chromosome VI

s3

Chromosome VII

nic8

Chromosome VIII



Figures represent recombination fractions. (%)

In bold type, mutants the location of which is the object of the present paper.

Symbols of mutant alleles:

Chromosome I.

su1ad2o = suppressor of *ad2o*. *ribo1* = riboflavine requirement.
ad14 = adenine requirement. *lu* = leucine requirement.
pro1 = proline requirement. *paba1* = *p*-amino-benzoica cid requirement. *y* = yellow coloured conidia. *ad2o* = partial adenine requirement. *bi1* = biotin requirement.

Chromosome II.

Acr1 = resistance to acriflavine (incompletely dominant).
w3 = white coloured conidia. *thi4* = thiazole requirement.
ni3 = nitrite requirement. *ab* = amino-butyric acid requirement.
ad1 = adenine requirement. *ad3* = adenine requirement.
an2 = aneurin requirement.

Chromosome III.

phen2 = phenylalanine requirement. *arg2* = arginine requirement.
meth2 = methionine requirement.

Chromosome IV.

meth1 = methionine requirement. *suc1* = inability to grow on sucrose as sole source of carbon. *pyro4* = pyridoxin requirement.

Chromosome V.

lys5 = lysine requirement.

Chromosome VI.

s3=sulphite requirement.

Chromosome VII.

nic8 = nicotinic acid requirement.

Chromosome VIII.

co = compact colony. *orn8* = ornithine requirement.

located by using both tester strains, is tentatively assumed to be in the remaining linkage group (VIII).

Tester Strain " A " has the following genotype :—

Chromosome I.	Chromosome II.	Chromosome III.
su y ad20	*Acr1*	*phen2*
Chromosome IV.	**Chromosome V.**	**Chromosome VII.**
pyr04	*lys5*	*nic8*

Tester Strain " B " :—

Chromosome I.	Chromosome II.	Chromosome VI.
su y ad20	*Acr1*	*s3*

Symbols and linkages are given in the table.

2. METHODS

For general techniques and symbols in the genetics of *Aspergillus nidulans* see Pontecorvo *et al.* (1953) ; Pontecorvo and Käfer (1958) and Käfer (1958).

For use in the technique described in this paper the most suitable genotype of a strain with an unlocated mutant, *x*, is *w ad20 x*. However, only a few strains in which an unlocated mutant is present have this genotype. The essential requirement is that *ad20* should be present with *x* ; the presence of *w* is desirable but not essential.

Dr E. Käfer (1958) suggested an easy way of introducing *ad20* into the strain with mutant *x*, provided the latter carries y^+. The essentials of the technique are as follows. A diploid between the strain with mutant *x* and the tester strain, *e.g.* A, is synthesised (Roper, 1952). This diploid will have the genotype :—

$$\frac{su \;\; y \;\; ad20 \;\; Acr1 \;\; phen2 \;\; pyr04 \;\; lys5 \;\; nic8}{+ \;\; + \;\; + \;\; + \;\; + \;\; + \;\; + \;\; +} \left(\frac{+}{x}\right)$$

The phenotype will be green (because y/y^+), partially resistant to acriflavine (because *Acr1*/+), and prototroph because heterozygous for all the nutritional markers. It will be also wild-type in respect of *x* if *x* is recessive.

As a first step in the analysis a yellow diploid segregant is selected by inspection from this green prototrophic diploid, and tested for adenine requirement. Mitotic crossing over anywhere between *y* and the centromere in a diploid $\frac{y \;\; ad20}{+ \;\; +}$ will give homozygous yellow diploid segregants which are also homozygous *ad20*, i.e. $\frac{y \;\; ad20}{y \;\; ad20}$. Thus, the genotype of this segregant will be the same as that of the parent diploid except that it is homozygous $\frac{y \;\; ad20}{y \;\; ad20}$.

The second step in the analysis is the selection of haploids from this yellow adenineless diploid segregant, twenty haploids being usually sufficient. To obtain these haploids we can apply the combined selection for adenine independence and for full acriflavine resistance. The third step is to classify haploids in respect of *x* and the markers on chromosomes III, IV, V and VII.

If *x* is, for example, on chromosome III, then only the two parental classes of haploids will arise in respect of *x* and *phen2*, i.e. *x* $phen2^+$ and x^+ *phen2*. If *x* is on chromosome pairs IV, V or VII, similarly only the parental classes will arise in respect of *x* and the marker on one of these chromosome pairs. If *x* is on chromosome pairs I or II, only x^+ will appear among the selected haploids. If *x* recombines

with all markers on chromosome pairs I, II, III, IV, V and VII carried by Tester Strain A, then the use of Tester Strain B will determine if *x* is on chromosome pair VI. If not, we tentatively conclude that it is on chromosome pair VIII.

As mentioned above, if *x* is on chromosome pairs I or II, it fails to appear among the selected haploids. We must then find on which of these two pairs it is located. Here the additional presence of *w* in the strain carrying *x* is particularly useful. In this case the yellow segregant first isolated will be $\frac{su\ y\ ad20}{+\ y\ ad20}\ \frac{Acr1\ +}{+\ w}$, and *x* will segregate with either $\underline{+\ y\ ad20}$ or $\underline{+\ w}$. Combined selection of $\underline{su\ y\ ad20}\ \underline{+\ w}$ haploids will show which of the two pairs is involved. If all the haploids so selected are *x*, then *x* is on chromosome pair II. If all of them are x^+, then *x* is on chromosome pair I.

An unlocated mutant phenotypically indistinguishable from one of the markers in the tester strain, but not allelic to it, could be located only if it were on certain chromosome pairs. For instance, if *x* stood for a mutant determining phenylalanine requirement, not allelic to *phen2* but located on the same chromosome, all the haploids recovered from the homozygous $\frac{y\ ad20}{y\ ad20}$ diploid segregant would require phenylalanine due to their being either *x* or *phen2*. If *x* were on the chromosome pair marked in repulsion by *pyro4*, then all the $pyro4^+$ would require phenylalanine by virtue of carrying either *x* or both *x* and *phen2*, i.e. $\underline{pyro4^+\ x}\ phen2^+$ or $\underline{pyro4^+\ x}\ phen2$. If *x* were on the chromosome pair carrying $\underline{su\ y\ ad20}$ or on the chromosome pair carrying *Acr1*, using the combined acriflavine resistance and adenine independence selection, only x^+ would be recovered among the haploids, but half of those would still require phenylalanine by virtue of *phen2*. By selecting for white and adenine independence, however, if *x* were on chromosome pair II then all the haploids would require phenylalanine since *w* is in coupling with *x*.

This, then, leaves only chromosome pairs I, VI and VIII as alternatives, and chromosome pair VI can be tested by using Tester Strain B. If not located by Tester Strain B only two possibilities remain, *x* is on chromosome pairs I or VIII.

In order to test the validity of the technique before applying it to unlocated mutants, it was first used with a marker *orn4*, the location of which on chromosome IV was already known from analysis through sexual reproduction (Käfer, 1958).

3. EXPERIMENTAL

Pilot Test with orn4

The *orn4* mutant determines requirement for ornithine. A balanced heterokaryon was formed between *bi1* ; *orn4* and Tester Strain A. From this, the heterozygous diploid was isolated in the usual way (Roper, 1952) and purified by single conidium micromanipulation. A suspension of conidia from the diploid was then plated onto complete medium so as to give well-separated colonies, and from one of these colonies a homozygous yellow segregant was selected visually. This was then purified by micromanipulation of a single conidium. The strain was tested for ploidy and adenine requirement. A dilute suspension of conidia of the diploid segregant $\frac{y\ ad20}{y\ ad20}$ was next point-inoculated (about five conidia per inoculation, at twenty-six points on a Petri dish) onto minimal medium plus all the growth factors required by each of the parental strains except adenine and biotin (the segregant

now being homozygous biotin-independent). After 24 hours incubation small spidery colonies had developed with the characteristic sparse growth typical of *ad2o* on adenineless medium. A layer of the same medium plus acriflavine was then poured on top. Another 4 days incubation was necessary before vigorously growing, well-sporulating segregants had escaped from the mass of partially resistant hyphæ and had grown through the top layer of medium.

Thirty-four haploids were isolated by picking only one segregant from any one colony to minimise the incidence of repeated isolation of products of the same event. The haploids when fully tested fell into the following classes :

		phen2				*phen2*$^+$				
		lys5		*lys5*$^+$		*lys5*		*lys5*$^+$		
		nic8	*nic8*$^+$	*nic8*	*nic8*$^+$	*nic8*	*nic8*$^+$	*nic8*	*nic8*$^+$	
pyro4$^+$	*orn4*	0	■	4	1	3	■	■	2	18 } 18
	orn4$^+$	0	□	0	0	0	0	□	0	0 }
pyro4	*orn4*	0	0	0	□	□	□	□	□	0 } 16
	orn4$^+$	1	4	2	4	1	1	1	2	16 }
		1 6		6 5		4 4		4 4		
		7		11		8		8		
		18				16				

From the results it can be seen that *orn4* shows recombination with all the markers, except *pyro4*, and is therefore located on the chromosome pair marked by *pyro4*. The two recombinant classes, *pyro4 orn4* and *pyro4*$^+$ *orn4*$^+$, are absent. These are the classes expected to be missing if *orn4* and *pyro4* are linked, since they are in repulsion and haploidisation involves no crossing over. All the markers of the other chromosome pairs segregate independently of one another.

4. LOCATION OF NEW MUTANTS

(I) (suc1)

Mitotic Analysis. The unlocated mutant (suc1) determines the inability to grow on sucrose as a sole source of carbon (Roberts, unpublished). A strain *bi1* ; *w3* ; *suc1* was combined with Tester

Strain A. Selection on the homozygous $\frac{y\ ad20}{y\ ad20}$ recombinant was the same as in the previous example. Twenty-five haploids were isolated, fully tested and found to fall into the following classes :—

		phen2				*phen2*+				
		lys5		*lys5*+		*lys5*		*lys5*+		
		nic8	*nic8*+	*nic8*	*nic8*+	*nic8*	*nic8*+	*nic8*	*nic8*+	
pyro4+	*suc1*	2	1	1	4	2	1	4	1	16
	suc1+	0	0	0	0	0	0	0	0	0
pyro4	*suc1*	0	0	0	0	0	0	0	0	0
	suc1+	1	2	0	3	1	0	1	1	9

16 | 16

9

3 3 1 7 3 1 5 2

6 8 4 7

14 11

From these results it can be seen that *suc1* shows recombination with all the markers except *pyro4*. The two recombinant classes *pyro4*+ *suc1*+ and *pyro4 suc1* are absent. These are the classes expected to be missing if *suc1* and *pyro4* are linked and in repulsion.

Meiotic Analysis. From a cross *pro1 paba1 y bi1* ; *meth1 pyro4* × *bi1* ; *w3* ; *suc1* the following relevant results were obtained :—

	meth1	*meth1*+
suc1+	124	39
suc1	26	120

Recombination frequency 21 per cent.

	pyro4	*pyro4*+
suc1+	92	71
suc1	53	93

Recombination frequency 40 per cent.

(ii) (meth2)

Mitotic Analysis. The unlocated mutant (meth2) determines requirement for methionine. A strain *bi1* ; *meth2* was combined with

Tester Strain A. Selection on the homozygous $\frac{y\ ad20}{y\ ad20}$ recombinant was again for acriflavine resistance and adenine independence. Thirty-seven haploids were isolated and fully tested.

The haploids fell into the following classes in respect of the combinations of the unlocated mutant with each of the markers identifying the different chromosome pairs :—

	phen2	*phen2*$^+$
meth2$^+$	19	0
meth2	0	18

	lys5	*lys5*$^+$
meth2$^+$	8	10
meth2	11	[illegible]

	nic8	*nic8*$^+$
meth2$^+$	0	18
meth2	16	3

	pyro4	*pyro4*$^+$
meth2$^+$	9	9
meth2	9	10

The results indicate that *meth2* and *phen2* are on the same chromosome pair.

Meiotic Analysis. From a cross *bi1* ; *meth2* × *pro1 paba1 y* ; *phen2* the following relevant results were obtained :—

	phen2	*phen2*$^+$
meth2$^+$	37	17
meth2	13	31

Recombination frequency 32·5 per cent.

(iii) (orn8)

Mitotic Analysis. The unlocated mutant (**orn8**) determines requirement for ornithine. *bi1* ; *orn8* was combined with Tester Strain A and selection on the homozygous $\frac{y\ ad20}{y\ ad20}$ recombinant was again for adenine independence and acriflavine resistance. Twenty-three haploids were isolated and fully tested. The haploids fell into the following classes in respect of the combinations of the unlocated

mutant with each of the markers identifying the different chromosome pairs :—

	phen2	*phen2*$^+$
orn8$^+$	5	4
orn8	7	7

	lys5	*lys5*$^+$
orn8$^+$	4	5
orn8	6	8

	nic8	*nic8*$^+$
orn8$^+$	6	3
orn8	5	9

	pyro4	*pyro4*$^+$
orn8$^+$	3	6
orn8	5	9

The results indicate that *orn8* is not located on any of the chromosome pairs marked by Tester Strain A. Another diploid was therefore synthesised, combining *bi1* ; *orn8* with Tester Strain B and a homozygous $\frac{y\ ad20}{y\ ad20}$ recombinant isolated. The combined selection for adenine independence and acriflavine resistance was applied to this recombinant. Sixteen haploids were isolated, tested and classified as follows :—

	s3	*s3*$^+$
orn8$^+$	6	4
orn8	2	4

Clearly *orn8* is not located on any of the marked chromosome pairs and by elimination we may assume that it is on chromosome pair VIII.

Meiotic Analysis. From a cross *bi1* ; *orn8* $\times$ *paba y* ; *co* (the marker *co* marks chromosome VIII), the following relevant results were obtained :—

	co	*co*$^+$
orn8$^+$	50	0
orn8	0	62

Thus it is confirmed that *orn8* is on chromosome VIII and very closely linked to *co*.

(iv) (an2)

Mitotic Analysis. The unlocated mutant (an2) determines requirement for aneurin. A strain *bi1* ; *Acr1 w3* ; *an2* was combined with Tester Strain A. Selection on the $\frac{y\ ad20}{y\ ad20}$ recombinant was for white and adenine independence. (It will be noted that in this case the original diploid was homozygous *Acr1*/*Acr1*). Twenty-one haploids were isolated, tested and all found to require aneurin.

Since all the haploids required aneurin and were white, and white was in the parent strain requiring aneurin, it follows that they are both located on the same chromosome pair.

Meiotic Analysis. From cross *bi1* ; *Acr1 w3* ; *an2* × *y* ; *ad1* the following relevant results were obtained :—

	ad1	*ad1*$^+$
an2$^+$	28	19
an2	19	30

Recombination frequency 39·6 per cent.

(v) (arg2)

Mitotic Analysis. The unlocated mutant (arg2) determines a requirement for arginine. A strain *bi1* ; *arg2* was combined with Tester Strain A. Selection on the homozygous $\frac{y\ ad20}{y\ ad20}$ recombinant was for adenine independence and acriflavine resistance. Twenty-eight haploids were isolated and fully tested.

The haploids fell into the following classes in respect of the combinations of the unlocated mutant with each of the markers identifying the different chromosome pairs :—

	phen2	*phen2*$^+$
arg2$^+$	11	0
arg2	0	17

	lys5	*lys5*$^+$
arg2$^+$	5	6
arg2	7	10

	nic8	*nic8*$^+$
arg2$^+$	3	8
arg2	8	9

	pyro4	*pyro4*$^+$
arg2$^+$	7	4
arg2	9	8

These results indicate that *arg2* and *phen2* are on the same chromosome pair.

Meiotic Analysis. From a cross *bi1* ; *arg2* ×*ribo1 ad14 y* ; *phen2* the following relevant results were obtained :—

	phen2	*phen2*+
arg2+	46	27
arg2	16	40

Recombination frequency 30 per cent.

(vi) (lu)

Mitotic Analysis. The unlocated mutant (lu) determines requirement for leucine. A strain *bi1* ; *lu* was combined with Tester Strain A. Selection on the homozygous $\frac{y\ ad20}{y\ ad20}$ recombinant was for adenine independence and acriflavine resistance. Twenty-one haploids were isolated, tested and found to be all leucine independent. The mutant *lu* was therefore located on either chromosome pair I or on chromosome pair II. As *bi1* ; *lu* did not carry *w3*, the selection for white and adenine independence could not be made. This selection would have made it possible to confirm on which of these two chromosome pairs *lu* was located.

Meiotic Analysis. Two crosses were set up with the *lu* mutant. One cross was to a strain with chromosome I extensively marked and the other to a strain with chromosome II extensively marked. Meiotic analysis from the cross of *bi1* ; *lu* ×*ribo1 ad14 pro1 paba1 y* gave the following relevant results :—

	ad14	*ad14*+
lu+	63	14
lu	7	63

Recombination frequency 14·3 per cent.

	ribo1	*ribo1*+
lu+	49	14
lu	24	43

Recombination frequency 30 per cent.

The cross to the strain with marked chromosome II was therefore not analysed.

(vii) (ab)

Mitotic Analysis. The unlocated mutant (ab) determines requirement for alpha-amino-n-butyric acid. A strain *bi1* ; *ab* was combined with Tester Strain A. Selection on the homozygous $\frac{y\ ad20}{y\ ad20}$ recombinant

was for adenine independence and acriflavine resistance. Forty-two haploids were isolated and fully tested. All were amino-butyric acid independent. As in the case of the *lu* mutant, selection for white and adenine independence could not be made.

Meiotic Analysis. Two crosses were set up, one to a strain with chromosome I extensively marked and the other to a strain with chromosome II extensively marked. Meiotic analysis from the cross *bi1* ; *ab* ×*rib*o*1 bi1* ; *Acr1 w3 thi4 ni3 ad3* gave the following relevant results :—

	ad3	*ad3*+
ab+	46	16
ab	25	45

Recombination frequency 31 per cent.

	ni3	*ni3*+
ab+	52	10
ab	13	57

Recombination frequency 18 per cent.

5. CONCLUSIONS

Of the eight mutants located by means of the haploidisation technique described in this paper, only one was meiotically closely linked to the relevant marker in the tester strains.

Four of the other seven were loosely linked meiotically with the marker of the tester strain relevant in each case. The remaining three showed no meiotic linkage with the relevant marker, but did show it with other markers meiotically linked to the relevant marker. Thus the efficiency of the technique is such that an unlocated mutant can be assigned to the linkage group to which it belongs even though the marker identifying that linkage group and the unlocated mutant are more than fifty meiotic map units apart.

Two further improvements of the technique are possible :—

(1) The use of a single tester strain with at least seven of the eight chromosomes marked instead of two tester strains.

(2) The use for combined selection of two markers, one on each arm of the same chromosome instead of one on each of two non-homologous chromosomes. A new marker *x* on the homologue of the "selector" chromosome would show its location on that chromosome because only x^+ would appear among the selected haploids. With the technique used so far, on the other hand, the recovery of x^+ leaves two alternative locations, *i.e.* on either of the two chromosome pairs used for selection.

An example of (2) is the following :—

For the isolation of new mutants, a strain y^+ *ad2o* is used. The diploid combination of a new mutant and a tester strain will be

$$\frac{su}{+} \circ \frac{y \quad ad2o}{+ \quad ad2o} \quad \frac{+}{x}$$

Visual selection of yellow segregants on adenineless medium will yield haploids of constitution *su y ad2o.*

With the use of this modified technique (combined selection for two markers, one on each arm of the same chromosome) we might expect that among the selected segregants the proportion of diploids should be somewhat higher than in the case of combined selection for two markers one in each of two non-homologous chromosomes. This is because of the rare occurrence of non-disjunction in individual chromosome pairs (see Pontecorvo and Käfer, 1958, p. 89), a trouble not likely to reduce substantially the efficiency of the technique.

6. SUMMARY

1. A technique based on mitotic segregation has been devised for quickly and easily assigning new mutants to their linkage groups in *Aspergillus nidulans*.

2. The technique is based on the fact that strains of *A. nidulans* with diploid nuclei produce haploids vegetatively as a rare but regular event. As the process of mitotic haploidisation is known not to be associated with mitotic crossing over, the individual members of each chromosome pair segregate as whole units. Thus, among such haploids, markers on different chromosome pairs show free recombination, while markers on any one chromosome pair do not recombine.

3. Heterozygous diploids are prepared between a suitable strain carrying the new mutant and two tester strains, marking between them seven of the eight chromosome pairs. Haploid segregants are selected automatically by a number of devices which distinguish them from those segregants, still diploid, which arise by mitotic crossing over.

4. Seven new markers on five chromosome pairs have been located by this technique. Subsequent meiotic analysis has confirmed each of these locations.

Acknowledgments.—The author would like to express his thanks to Professor G. Pontecorvo, F.R.S., for his criticism and advice both during the work and, especially, in the preparation of this paper.

This work is part of a programme supported by the Nuffield Foundation.

7. REFERENCES

KÄFER, E. 1958. An 8-chromosome map of *Aspergillus nidulans*. *Adv. Genet.*, *9*, 105-145.

PONTECORVO, G., AND KÄFER, E. 1956. Mapping the chromosomes by means of mitotic recombination. *Proc. Roy. phys. Soc.* (Edin.), *25*, 16-20.

PONTECORVO, G., AND KÄFER, E. 1958. Genetic analysis based on mitotic recombination. *Adv. Genet.*, *9*, 71-104.

PONTECORVO, G., ROPER, J. A., HEMMONS, L. M., MACDONALD, K. D., AND BUFTON, A. W. J. 1953. The genetics of *Aspergillus nidulans*. *Adv. Genet.*, *5*, 141-238.

PONTECORVO, G., TARR-GLOOR, E., AND FORBES, E. 1954. Analysis of mitotic recombination in *Aspergillus nidulans*. *J. Genet.*, *52*, 226-237.

PRITCHARD, R. H. 1955. The linear arrangement of a series of alleles of *Aspergillus nidulans*. *Heredity*, *9*, 343-371.

ROPER, J. A. 1952. Production of heterozygous diploids in filamentous fungi. *Experientia*, *8*, 14-15.

ROPER, J. A., AND KÄFER, E. 1957. Acriflavine resistant mutants in *Aspergillus nidulans*. *J. Gen. Microbiol.*, *16*, 660-667.

A CYTOPLASMICALLY CONTROLLED ABNORMALITY OF THE TETRADS OF *COPRINUS LAGOPUS*

P. R. DAY

John Innes Horticultural Institution, Bayfordbury, Hertford, Herts

Received 30.v.58

Among the gill-bearing forms of the higher basidiomycetes (Agaricacæ) meiosis in the basidium is generally followed by the production of four haploid basidiospores in a tetrad. Some Agaricacæ have two-spored basidia such as the two-spored forms of species of the genus *Mycena* (Smith, 1934) or the cultivated mushroom *Psalliota campestris*. Examples of basidia bearing one, three, five, six or more basidiospores appear to be less common but they have been reported in *Psalliota campestris* (Kligman, 1943) and *Coprinus* species (Buller, 1922, 1931, and Sass, 1932).

Tetrads with more or less than the normal number of four spores were found on the gills of some fruit bodies from crosses of two wild-type monocaryons of *Coprinus lagopus* (Day, 1957). This paper describes an investigation of this abnormality.

1. METHODS

(i) *Media*

Monocaryons and dicaryons were grown on a complete medium made up from Fries (1953) medium: L-asparagine, 2·0 g.; NH_4 tartrate, 1·5 g.; KH_2PO_4, 1·0 g.; Na_2HPO_4, 2·25 g.; Na_2SO_4, 0·29 g.; thiamin, 40 μg.; glucose, 20·0 g.; agar, 20 g.; water to 1000 ml. supplemented with Oxoid yeast extract, 0·75 g.; bacteriological peptone (Eupepton No. 2, supplied by Messrs Allen & Hanbury, Ware, Herts), 0·75 g. and Oxoid malt extract, 0·6 g.

Fruit bodies were produced on horse dung which had been moistened and autoclaved for half an hour in half-pint milk bottles closed with aluminium foil. They appeared from 7-10 days after inoculation with a dicaryon.

All cultures were incubated at 28° C. in daylight, in a glass-fronted incubator.

(ii) *Stocks*

Two basidiospores were isolated by Professor D. Lewis from a single fruit body occurring on a manure heap at Barwick Ford, Herts, in 1952. The cultures they gave rise to were numbered 54 and 68 and since they were compatible they were given the mating types A_1B_2 and A_2B_1 respectively.

Stock cultures were maintained under mineral oil at room temperature and at 4° C.

(iii) *Crosses*

When two haploid, compatible monocaryons are crossed inocula of each are placed 2-4 mm. apart on complete medium in a petri dish. After 2-3 days' incubation both monocaryons may become dicaryotised, when the vigorous hyphæ with clamp connections can easily be recognised. In describing a cross, the monocaryon which is the source of the dicaryon is written first although, of course, dicaryotic mycelia can be taken from either parent.

(iv) *Cytology*

To study meiosis in the basidia fruit bodies at various stages of development were fixed in 1 : 3 acetic alcohol for half an hour and stored in 70 per cent. (v/v) alcohol at 4° C. Fragments of gill tissue were transferred directly to cold IN HCl for 5 minutes and then to IN HCl at 60° C. for 5 to 7 minutes. After rinsing in distilled water the fragments were tapped out in a drop of a freshly-diluted and filtered solution of synthetic orcein (1 ml. 5 per cent. (w/v) stock solution in glacial acetic acid + 1 ml. distilled water). Pressure was applied through two layers of filter paper to a cover slip placed over the drop and, after heating to improve differential staining, each slide was sealed with paraffin wax and stored at 4° C.

Other methods are described where relevant in the text.

2. RESULTS

In January 1957 the dicaryon $68(A_2B_1) \times 54(A_1B_2)$ gave rise to normal fruit bodies. The gill surfaces of a normal fruit body are black with densely-crowded basidiospores in tetrads. Before maturity the gills are white and there is a stage when each fully-formed basidiospore is colourless. However, as it reaches maturity, the deposition of a black pigment begins at the end of the spore distal to the sterigma and continues until the entire spore wall is darkly pigmented. The density of tetrads is about 3000 per sq. mm. of gill surface (plate, fig. 2).

At the same time the reciprocal dicaryon $54(A_1B_2) \times 68(A_2B_1)$ gave rise to abnormal fruit bodies with pale almost white gills at maturity (plate, fig. 1). The tetrad density on the gills of these fruit bodies ranged from 0-600 per sq. mm., the denser areas occurring in patches. Among the mature tetrads were basidia arrested at various stages of development : some were completely sterile, others produced small sterigmata or spore rudiments. Some of the mature " tetrads " were abnormal, having two, three, five or six spores (plate, figs. 3 and 4). One basidium was seen with eight spores. The distribution of these abnormal tetrads was non-random. Certain areas of a gill produced mainly threes and fours and other areas mainly fours and fives. Abnormal tetrads occasionally occurred in pairs. Estimates of the relative frequencies of the different types of tetrad were made by counting 2000 tetrads of a typical pale fruit body. These are given in table 1. The relative frequencies varied between fruit bodies and between gills within a fruit body. Thus, although the average frequency of abnormal tetrads from table 1 is 12·9 per cent., patches of the gills of some pale fruit bodies had frequencies as high as 40 per cent.

Twenty-five abnormal tetrads were isolated with a micromanipulator and transferred to complete medium. In no case did all the spores of a tetrad germinate, whereas up to 75 per cent. of tetrads from normal fruit bodies germinate completely. The germination results are given in table 1. Two pentads which each gave rise to four colonies are of special interest. The colonies were tested for mating type with tester stocks $C527(A_3B_1)$ and $C728(A_2B_4)$. The first set of four was

parental ditype for A and B alleles ($2A_1B_2 : 2A_2B_1$), the second set was tetratype except that one class (A_1B_2) was missing and another (A_1B_1) was represented twice ($2A_1B_1 : A_2B_1 : A_2B_2$). Among the

TABLE 1

Abnormal tetrad frequencies and spore germination of a pale fruit body of the dicaryon $54(A_1B_2) \times 68(A_2B_1)$

Tetrad spore no.	Per cent.	No. of tetrads isolated	Spores germinated per tetrad
6	0·2	1	0
5	4·5	2	4*
		13	3 or less
4	87·1	0	...
3	7·9	3	■
		4	1
		1	0
2	0·3	1	1

* Mating types :—$2A_1B_1$, $2A_2B_2$ (non-parental ditype).
$2A_1B_1$, A_2B_1, A_2B_2 (tetratype ?).

other cultures from incomplete sets, dwarfed, gnarled mycelia were common.

Several dicaryons were produced by crossing compatible monocaryons with normal vigour derived from basidiospores produced by pale fruit bodies. All gave rise to normal fruit bodies.

TABLE 2

Distribution of nuclei and sterigmata in the basidia of an immature pale fruit body from the dicaryon 54×68

Sterigmata	Nuclei						Total	Per cent.
	2	3	4	5	6	7		
2	1	0	2	0	0	1	4	3·2
3	1	6	7	4	2	0	20	16·0
4	4	7	81	4	2	1	99	79·2
5	0	0	2	0	0	0	2	1·6
Total .	6	13	92	8	4	2	125	...
Per cent. .	4·8	10·4	73·6	6·4	3·2	1·6	...	...

Acetic-orcein preparations of gill fragments from young fruit bodies produced by the dicaryon 54×68 showed that meiosis in many basidia was delayed or arrested at M1. Stages after M1 often showed bridges, chromosome fragments and micronuclei. At stages after T2 departures from the normal number of four nuclei were common. The number of nuclei and sterigmata for each of 125 basidia were scored in a preparation made from a fruit body with immature tetrads. The results are given in table 2. Eighty-one basidia (64·8 per cent.) were normal with four sterigmata and four nuclei.

Corner (1948) has suggested that the sterigmata and basidiospores arise independently of the nuclei and centrosomes of the basidium. If the sterigmata were organised by the nuclei there would be as many sterigmata as nuclei. There might be fewer sterigmata in abnormal fruit bodies because of nuclear unbalance brought about by aborted meiosis, but there could never be more sterigmata than nuclei. Table 2 shows that fourteen basidia (11·2 per cent.) had more sterigmata than nuclei. Some of these could be explained by loss of nuclei through the sterigmata during the preparation of the slide. However, in five instances all the rudimentary spores were still attached.

The reciprocal crosses were repeated three months later on a larger scale using subcultures 54′ and 68′ of the original stocks but the differences were not as extreme as previously. The dicaryon 54′ ×68′ gave pale fruit bodies but 68′ ×54′ gave normal and pale fruit bodies. Some of the pale fruit bodies had small sectors of dark gills with normal tetrad densities and few abnormal tetrads. These were separated by larger sectors of pale gills with the usual patchy distribution of normal and abnormal tetrads (plate, fig. 6).

Subcultures of the original dicaryon 68 ×54 which had given normal fruit bodies gave pale as well as normal fruit bodies. These occurred together in the same flask and in one instance a fruit body of each type arose from adjacent centres. A dicaryon isolated from the stipe of the pale fruit body gave rise to pale fruit bodies when inoculated to dung. A similar dicaryon isolated from the stipe of the normal fruit body gave rise to normal fruit bodies.

Normal and pale dicaryons were resolved into their component monocaryons by using a method developed by Lewis (unpublished). A chlamydospore (plate, fig. 5) suspension is obtained by crushing aerial mycelium scraped from the surface of a 10-14-day-old culture of a dicaryon, in sterile water. The suspension is filtered through glass wool to remove debris, plated and incubated for 24-36 hours. Many chlamydospore germ tubes and resulting hyphæ bear clamps but a proportion have no clamps. The hyphal tips without clamps are cut off to include 2-5 cells and transferred to slants when a variable proportion give rise to monocaryons. The nuclei of these cultures can be identified by mating tests with the two parental cultures.

Three dicaryons were resolved in this way. They were :

1. the stipe subculture of the original dicaryon 68 ×54 which gave normal fruit bodies,
2. the stipe subculture of the same dicaryon which gave pale fruit bodies,
3. 54′ ×68′ giving pale and some sectored pale fruit bodies.

The resulting monocaryons were identified by mating tests with 54′(A_1B_2) and 68′(A_2B_1). Representative monocaryons from 1 and 2 were used in crosses and the resulting dicaryons inoculated to dung to determine fruit body type. The results are shown in table 3.

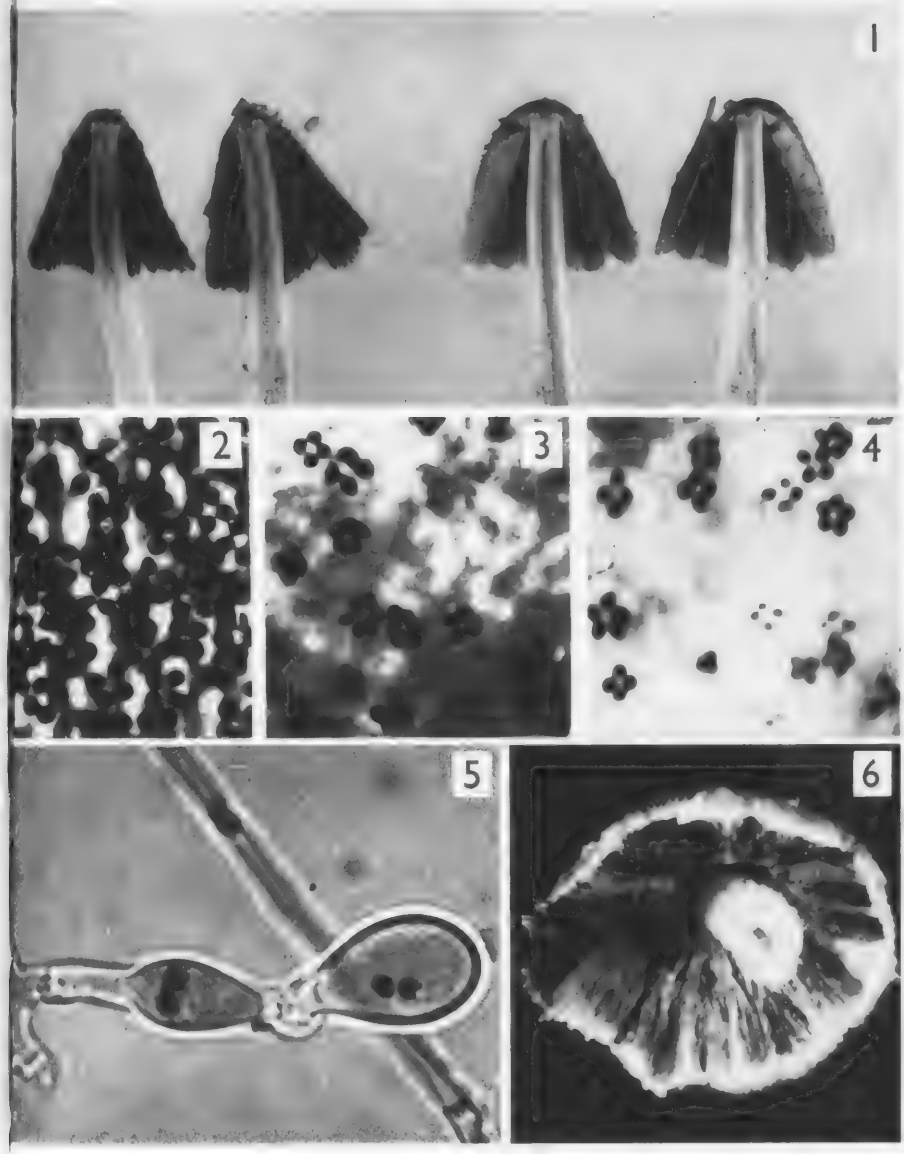


FIG. 1.—Left ; Normal fruit body from 68×54.
Right ; Pale fruit body from 54×68.
Both fruit bodies split longitudinally. *c.* natural size.

FIG. 2.—Gill surface of normal fruit body. *c.* ×450.

FIGS. 3 and 4.—Gill surfaces of pale fruit body—normal and abnormal tetrads. *c.* ×450.

FIG. 5.—Chlamydospores of a dicaryon stained with acetic-orcein. *c.* ×2000.

FIG. 6.—Sectored pale fruit body viewed from below. ×3.

Four of the eleven dicaryon cultures produced by single chlamydospores from 68 ×54 (normal) were fruited. Three gave pale fruit bodies and one gave normal fruit bodies. Eight dicaryons from 68 ×54 (pale) chlamydospores all gave rise to pale fruit bodies only.

TABLE 3

Results of resolving three dicaryons into their components

Dicaryon	No. of isolates	Dead	Dicaryons	68 nucleus	54 nucleus	?
(1) 68 ×54 normal .	59	6	11	28	14	...
(2) 68 ×54 pale . .	34	1	15	7	7	4
(3) 54′×68′ pale . .	45	5	18	13	6	3

Fruit body types produced by crossing component monocaryons

Cytoplasm parent	Other parent	Fruit bodies
54 (ex normal dicaryon)	×68′	normal
54 (ex pale dicaryon)	×68′	pale (some sectored)
68 (ex normal dicaryon)	×54 (ex pale dicaryon)	pale
68 (ex pale dicaryon)	×54 (ex pale dicaryon)	pale
68 (ex pale dicaryon)	×54 (ex normal dicaryon)	normal
54 (ex pale dicaryon)	×68 (ex normal dicaryon)	pale

3. DISCUSSION

When two compatible haploid monocaryons of *Coprinus lagopus* are crossed the two mycelia grow and, when they touch, exchange nuclei. The monocaryon growth of each inoculum gives way to dicaryon growth. Since reciprocal dicaryons contain the same nuclei, differences between them must reflect differences between the cytoplasms of the parental monocaryons. It is justifiable to assume that the monocaryon which receives the migratory nuclei will contribute the bulk of the cytoplasm to the resulting dicaryon.

The initial difference between the reciprocal dicaryons 68 ×54 and 54 ×68 suggests a cytoplasmic difference between the two stocks. The deterioration of the 68 monocaryon and the normal dicaryon suggest that the cytoplasmic change may be a result of ageing. The occurrence of both types of fruit bodies from a common inoculum and the occurrence of sectored fruit bodies demonstrate segregation of the cytoplasmic elements. The results from resolving dicaryons show that in the main the components follow the cytoplasmic character of the dicaryon parent. The exceptions are most likely due to variation between the samples of cytoplasm contained in chlamydospores from a common mycelium. The variation between four dicaryons which originated as chlamydospores of the dicaryon 68 ×54 (normal) is evidence of this.

It seems likely that the primary effect of abnormal cytoplasm is on meiosis, leading to abnormal divisions in many basidia. The isolated tetrads show the reduced viability and stunted mycelia expected from chromosomal unbalance due to aborted meiosis. One tetrad points to premature mitosis as another effect. The unusual tetrad $2A_1B_1$, A_1B_2, A_2B_2 from a set of five spores can be most easily explained by assuming that it was tetratype and that a mitotic division of the A_1B_1 nucleus took place in the basidium and the missing nucleus (A_2B_1) was lost in the spore that did not germinate. In a normal fruit body mitosis occurs after each nucleus has migrated into a basidiospore. Premature mitosis in the basidium would contribute to the classes in table 2 with more than four nuclei. A third effect of abnormal cytoplasm is that on the organisation of sterigmata and basidiospores so that departures from the normal number are common.

In *Coprinus sterquilinus* (Sass, 1935) aborted meiosis and abnormal tetrads have been brought about by overcrowding in the fruiting cultures. It seems unlikely that overcrowding is responsible for the abnormalities described here since single pale fruit bodies have been frequently seen in the culture flasks as well as fruit bodies of all three types ; normal, sectored and pale.

4. SUMMARY

Fruit bodies with pale gills bearing tetrads with from two to six basidiospores were produced by crossing two wild-type stocks. The reciprocal cross gave normal fruit bodies. When the parent stocks were subcultured and the crosses repeated three months later, both gave rise to abnormal fruit bodies although some from the cross which had previously been normal had normal sectors.

Normal and abnormal dicaryons were resolved into their components and these, when used as cytoplasm parents in further crosses, with some exceptions, followed their parent dicaryons in the type of fruit body produced.

A cytological examination of abnormal fruit bodies revealed irregularities in the meiotic divisions in the basidia producing bridges and micronuclei. There was no correlation between the number of nuclei and sterigmata in abnormal basidia.

Acknowledgments.—I am indebted to Professor D. Lewis, F.R.S., for his helpful discussion and encouragement. I also wish to express my appreciation of Mrs Margaret Lamdin's assistance.

5. REFERENCES

BULLER, A. H. R. 1922. *Researches on Fungi.* Longmans Green, London. Vol 2, p. 492.

BULLER, A. H. R. 1931. *Researches on Fungi.* Longmans Green, London. Vol. 4, p. 329.

CORNER, E. J. H. 1948. Studies in the basidium. I. The ampoule effect, with a note on nomenclature. *New Phytol.*, *47*, 22-51.

DAY, P. R. 1957. Abnormal tetrads in *Coprinus lagopus*. Abstr. *Heredity*, *11*, 426.

FRIES, L. 1953. Factors promoting growth of *Coprinus fimetarius* (L) under high temperature conditions. *Physiol. Plant.*, *6*, 551-563.

KLIGMAN, A. M. 1943. Some cultural and genetic problems in the cultivation of the mushroom, *Agaricus campestris* Fr. *Amer. J. Bot.*, *30*, 745-763.

SASS, J. E. 1932. The cytology of a diploid sterile hymenomycete. *Mycologia*, *24*, 229-232.

SASS, J. E. 1935. Cytological aspects of physiological sterility in *Coprinus sterquilinus*. *Ann. Bot.*, *49*, 151-154.

SMITH, A. H. 1934. Investigations of two-spored forms in the genus, *Mycena*. *Mycologia*, *26*, 305-331.

PARALLEL POLYMORPHISM IN THE HETEROCHROMATIN OF *TRILLIUM* SPECIES

C. D. DARLINGTON and G. W. SHAW
Botany School, Oxford

Received 3.vi.58

1. INTRODUCTION

THE mechanisms of heredity are similar in all organisms but the mechanisms of variation are widely different. The study of chromosome structure, especially of heterochromatin, reveals the differences and helps us to understand them.

The ten chromosomes of *Trillium* contracted at mitosis are a quarter of a millimetre long. They are also marked by larger segments of heterochromatin than any other plants. These H-segments, to use Rutishauser's abbreviation, are groups of genes which differ from the rest in two respects. They remain condensed within the resting nucleus; and they are exactly recordable at metaphase following cold treatment. This is due to their deficiency in DNA which is shown by a weak Feulgen reaction and a narrower chromatid (Darlington and La Cour, 1941, Shaw, 1958, 1959, Heyes and Shaw, 1958).

Variations occur within the species, and indeed within populations, in the numbers, sizes and positions of H-segments. The variations are discontinuous so that the species may be described as polymorphic in the sense of Ford. The range of variation within populations can then be compared among species. Homozygotes and heterozygotes in respect of every difference can be distinguished and counted. By this means the character of polymorphism in heterochromatin may be compared with that shown in other organisms at both the chromosome and the phenotypic levels.

2. MATERIALS AND METHODS

Ten species of *Trillium* and one species of *Paris* have been used (table 1). Their naming was confirmed with the help of Bailey (1941), Gray (1950) and Britton and Brown (1952).

It is important to know for our purpose how far these systematists' species are genuine units, genetically isolated from one another, not interbreeding in nature and therefore not overlapping in variation either at the chromosome or the phenotypic level. Our smaller collections do not enable us to be certain of the answer to this question. Our larger collections, however, indicate that *T. luteum* and *T. sessile* may be only colour polymorphisms within a genetic species governed by a supergene difference. The same kind of distinction may exist between *T. erectum* and *T. cernuum*.

Our genuine species may thus be less than ten. On the other hand we are confident that the three main sub-divisions into which we have divided the species in table 10 are absolutely isolated: making the most conservative estimate, as we must in arriving at our conclusions, our plants are of three species. On a reasonable estimate they are eight.

Geographical Distribution. The American species of *Trillium* are in two groups. The western species (*T. chloropetalum* and *T. ovatum*) are confined to the coast states. The eastern species range from Nova Scotia and Minnesota to Florida and Arkansas. There appears to be more heterochromatin in those species with the more northerly range. *T. stylosum* with least heterochromatin has a limited distribution which is also the most southerly.

Nomenclature. Three plants named *T. stylosum* and five named *T. grandiflorum* (3-7) by Darlington and La Cour correspond with a pattern we now recognise (by the D) as belonging to *T. recurvatum.* Some plants named *T. grandiflorum* by P. C. Bailey (1954) on the other hand (by A and B) seem to be *T. erectum.* Bailey's single

TABLE 1

Species	Chromosome no. ($x = 5$)	No. of plants	Source	References
EASTERN AMERICAN				
T. erectum	2*x*, 0-6*ff*, 3*x*	70	Messrs Perry	2, 3, 4, 8
T. cernuum	2*x*, 0-2*ff*	45	,,	9
T. luteum	2*x*, 0-2*ff*	30	,,	5
T. sessile	2*x*, 0-3*ff*	20	,,	2, 5
T. grandiflorum	2*x*, 0-2*ff*	65	,,	2, 6, 10, 11
T. stylosum	2*x*	20	,,	None
T. undulatum	2*x*	10	,,	3
T. recurvatum	2*x*	1	Oxford Bot. Garden	2
WESTERN AMERICAN				
T. ovatum	2*x*, *f*	1	Edinburgh*	None
T. chloropetalum	2*x*	3	Worcester*	None
HIMALAYAN				
Paris polyphylla	2*x*, 0-1*f*	8	Edinburgh*	1, 7
Total plants examined : 273				

1. Darlington and La Cour, 1938.
2. Darlington and La Cour, 1940.
3. Wilson and Boothroyd, 1941, 1944.
4. P. C. Bailey, 1949.
5. P. C. Bailey, 1952.
6. P. C. Bailey, 1954.
7. La Cour, 1951.
8. Boothroyd, 1953.
9. Rutishauser and La Cour, 1956.
10. Giles and Wilson, 1956.
11. Rutishauser, 1956*b*.

* We are indebted to Captain R. G. Berkeley of Spechley Park, Worcester, and the Director of the Royal Botanic Garden, Edinburgh, for gifts of plants of these species. Perry's seven eastern species were collected by Mr E. C. Robbins of MacDowell Co., in the west of North Carolina, the common ground of these species.

plant named *T. recurvatum* with only a single H-segment (terminal in B) does not correspond with any combination we have yet found in a single individual.

The five pairs of chromosomes of *Trillium* seem to correspond in all species and are labelled A, B, C, D and E (figs. 1-8).* The European system which we shall follow has been to label the chromosomes A to E in descending order of size from 36 μ to 22 μ. The American and Japanese equivalents we assume are given below :

Eur	A	B	C	D	E
Jap	A	C	B	E	D
Am	E	B	D	C	A

* In one instance only (table 6, footnote), the distinction between C and D was uncertain and interchange could have taken place. Interchange in evolution is, however, effectively absent. This is not surprising since we know that in *Paris* there is proximal localisation of chiasmata such as would sterilise interchange heterozygotes (Darlington, 1941).

Supernumerary chromosomes (f) are of two types: subterminals (s^1, s^2), and telocentrics (t^0, t^1, t^2).

In tables 2-9 each variant of a chromosome type has been given a number from 1 to 14. When a chromosome is homozygous, the number is given once only. When heterozygous, both numbers are given.

Where the mitotic complements of two or more plants in a species correspond exactly in their H-segment pattern, and hence are cytologically indistinguishable, the plants are provisionally assumed to belong to a single clone. The analysis of the plants examined is given in terms of these clones in tables 2-9.*

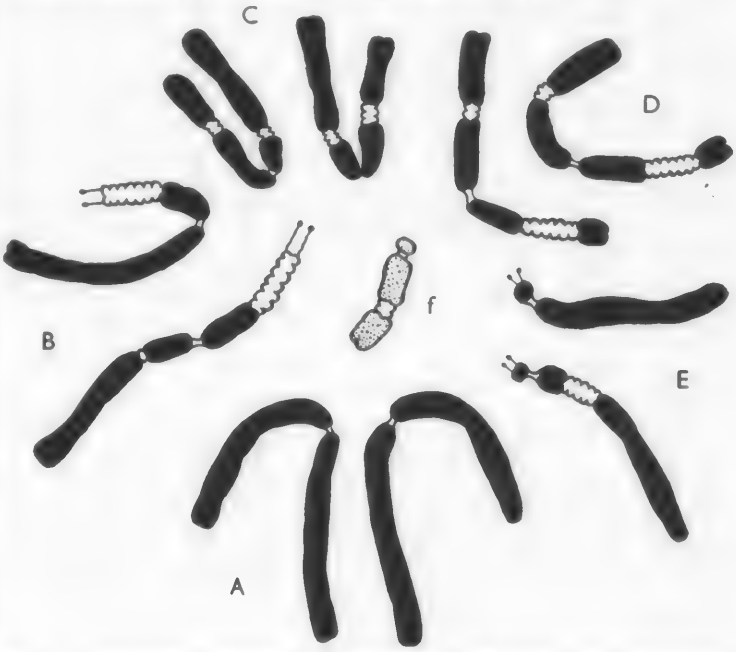


FIG. 1.—Chromosome complement of *T. ovatum* from root showing twelve DNA-starved H-segments.

Formula: A1.1, B9.14, C7, 7, D5′.5′, E1.2, f^h or s^2.

Note.—B and E have nucleolar satellites; these are specific to their own loci in each species but are not recorded in our formulæ and diagrams since they are usually collapsed in other species. B and E happen to be the only heterozygous chromosomes in this complement. See figs. 6 and 10. × 1800.

Techniques. Plants were kept at 1°-2° C. for 96 hours before fixation. Where chromosomes showed signs of stickiness, a further 24 hours in colchicine (0·01 per cent.) at 1° C. was given before fixation.

Root tips were taken from potted plants and fixed one hour in 2BD, hydrolysed for 16 minutes at 60° C. in N.HCl, and stained one hour in Feulgen solution. Squash preparations were dehydrated in Cellosolve (Shaw, 1950, 1956), cleared in xylene and mounted in Canada balsam.

* We do not believe this assumption to be true since the multiple clones assumed on this rule are those having the commonest chromosome types. This is, however, the most modest assumption in relation to our arguments since it underestimates heterozygosity.

3. CHROMOSOME COMPLEMENTS OF THE SPECIES

1. *Trillium erectum* (28 clones). Two chromosome types new for the genus were found in this species. B 12 occurred in 19 of the 28 clones. E 7 was in a single triploid all of whose chromosomes were homozygous. The triploidy did not modify the differential reaction of the H-segments.

The novel type of chromosome in the triploid, the first found among American populations, is an instance of the principle that polyploids, owing to their selective

FIGS. 2-6.—Diagrams of complements in ten species of *Trillium* levelled at the centromeres: showing all the types of each chromosome found in each species. × *c.* 1200.

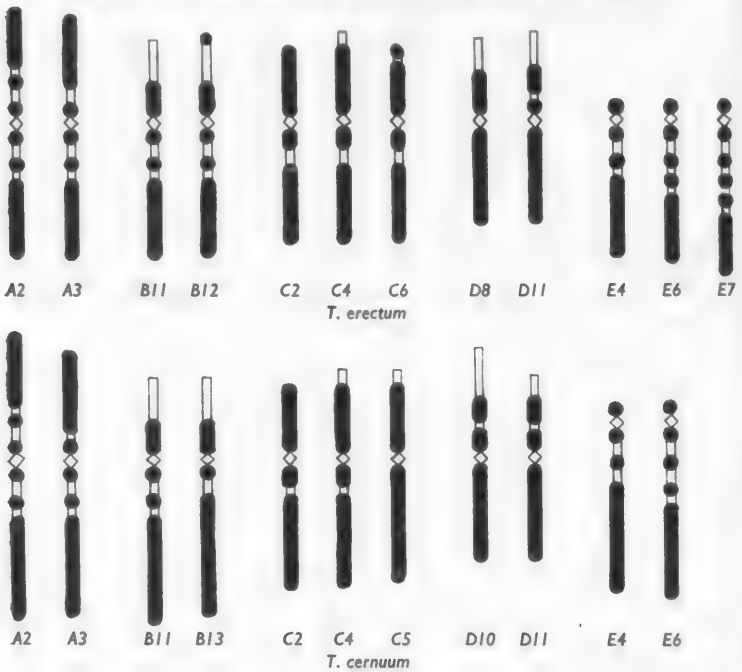


FIG. 2

survival, are unrepresentative of the diploid populations from which they have arisen.

More H-segments are illustrated by Wilson and Boothroyd (1941, 1944) than we or other workers have found. Their photographs suggest artefacts.

1. *T. kamtschaticum* as described by Darlington and La Cour seems to correspond to our *T. erectum*.

2. *T. cernuum* (22 clones). Like *T. erectum* this species has a proximal H-segment in each arm of A and in the long arm of B. These segments distinguish the two species from all the rest. Our plants contain more H-segments than those of Rutishauser and La Cour (1956).

3. *T. luteum* (28 clones). The terminal H-segment on B occurs in a series of lengths (0, 1·5 μ, 3 μ, 4·5 μ and 6 μ). The intercalary H-segment in C also occurs in two lengths.

Bailey's (1952) plant would appear to have the formula A1.B8.C4.D5.E1. (homozygous).

4. *T. sessile* (13 clones). All the 11 types of chromosome found in this species, except C1, occur also in the larger sample of *T. luteum*.

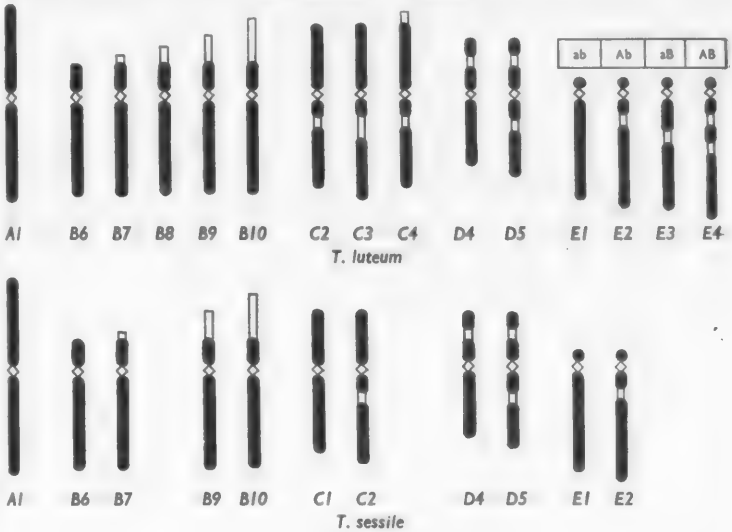


FIG. 3

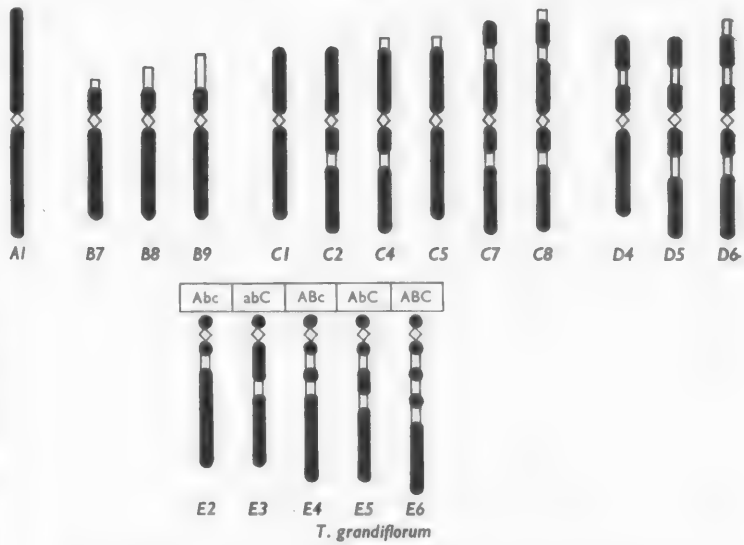


FIG. 4

5. *T. grandiflorum* (31 clones). Our findings agree with those of other authors. In the root tips of our 65 plants A is unvarying with no H-segments. In endosperms,

where the coiling is not so compact as in the root, some plants have the small proximal H-segment in the long arm of A also noticed by Rutishauser.

6. *T. stylosum* (10 clones). This species has the smallest proportion of heterochromatin (*c.* 2 per cent.). It is distinct from the other species only in the B and D

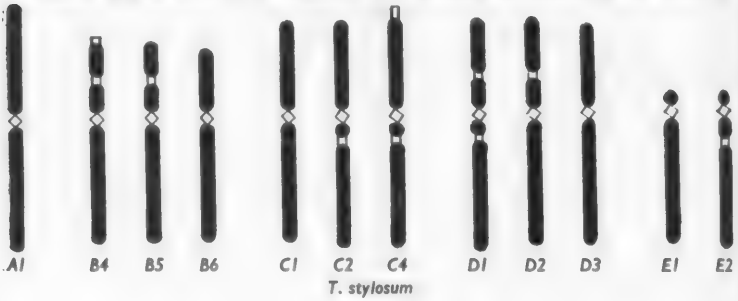


FIG. 5

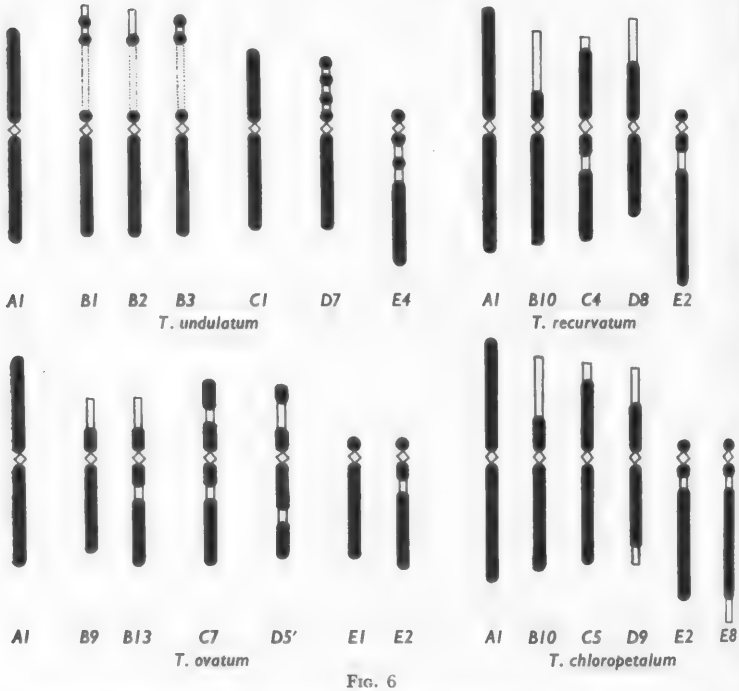


FIG. 6

chromosomes which are those developing most heterochromatin in the other species. In one plant (clone 1) unique in the genus, no H-segments could be seen after cold treatment; two tiny chromocentres were detected in the resting nuclei.

7. *T. undulatum* (3 clones). In no other American species do the H-segments reach such a high degree of differential starvation. Chromosome B has a proximal

segment in the short arm which can be seen as a starved H-segment even in unchilled metaphases. When plants are chilled, this segment fails to spiralise, and can reach up to five times its unchilled length. The length varies, depending no doubt upon how much it is stretched by movements in the cell. A non-spiralising H-segment was first found in the 8x*Paris japonica*. It is characteristic of species like *T. undulatum* with a low H-content (Darlington and La Cour, 1940).

The distal largely euchromatic region of this arm is the sole variable in the complement of the species. It also shows the only variation that we have seen in the euchromatin of *Trillium*. Chromosome D7 is peculiar to this species.

8. *T. ovatum*. Our single clone was heterozygous in B and E. It was homozygous for $D5^1$ a modification of D5 with a lengthened H-segment.

9. *T. recurvatum*. Our single clone agrees with the earlier account except in being homozygous. It reaches the maximum H-content of 25 per cent.

10. *T. chloropetalum* (2 clones). The H-pattern differs in D and E from all *Trillium* species and resembles *P. polyphylla*. The species is thus intermediate between the two genera. In the endosperm of a species cross a D chromosome revealed a small intercalary H-segment in each arm. One of our three plants was heterozygous : E2.8.

11. *Paris polyphylla* (2 clones). This species and its relatives, like *Trillium kamtschaticum*, have two chromosomes D and E with subterminal centromeres, and also several proximal H-segments. These two chromosomes are also parallel in their H-pattern but whereas the H-segments in all chromosomes are distal in *Paris* they are proximal in the Japanese *Trillium*.

Our two new clones were both different from the earlier ones although all came from Edinburgh (fig. 7).

4. CHROMOSOME SPIRALISATION

The chromosomes of all our species of *Trillium* are approximately the same size at metaphase of mitosis at normal temperatures. Those with H-segments are of course proportionately smaller when chilled and starved. To determine the proportion of heterochromatin we need, however, to measure relative lengths which are characteristic for each clone. Now, the absolute length svary from one cell to another with the degree of spiralisation which has followed differences in the prolongation of the metaphase by colchicine. We have therefore had to calibrate our observations of different clones and species.

How was this to be done? We havefound that the lengths of euchromatin are strictly in proportion in the corresponding chromosomes of the different species. It was not difficult to find cells which closely corresponded in all species as shown in figs. 1-8. From these the composite diagram in table 10 has been derived. From these also the proportions of starved heterochromatin in table 11 have been calculated. It should be noted, however, that in the starved chromosomes the euchromatin is a little over-spiralised and the heterochromatin a little under-spiralised. As in the comparable estimate of La Cour (1951), therefore, the proportion of the unstarved length of heterochromatin would be smaller than that calculated from the starved chromosomes.

Between the American and Japanese species of *Trillium* a notable difference lies in the eight non-spiralising segments of *T. kamtschaticum* and its hybrids (Haga, 1956). Only one such has been found in one

Tables 2-9.—Clonal analysis of eight species in respect of H-segment types (1-13) and heterozygosity of the five chromosomes (A-E) and variation in supernumeraries (f) of six types (s^1, s^2, t^0, t^1, t^2, t^3). H and L mark the clones with highest and lowest H-segment content.

TABLE 2

T. erectum (*70 Plants : 28 Clones*)

Clone no.	Chromosome type and variant					No. of plants		No. and type of *f*
	A	B	C	D	E	No *f*	With *f*	
				Homozygous				
1	2	11	2	8	4	*4*	1	t^1
2	2	11	2	11	4	*8*	...	...
3	2	11	2	11	6	*1*	...	...
4	2	11	6	8	4	*5*	...	...
5	2	11	6	8	6	*2*	...	...
6	2	11	6	11	4	*1*	...	...
7	2	11	6	11	6	*1*	...	...
8	2	12	2	8	4	*3*	...	...
9	2	12	2	8	6	*1*	...	...
10	2	12	2	11	4	*2*	2	$2t^1$
11	2	12	2	11	6	*4*	1	$4t^0$
12	2	12	4	8	4	*2*	1	s^1
13	2	12	4	11	4	*5*	...	H ...
14	2	12	6	8	4	*1*	1	$6t^0$
15	2	12	6	11	4	*1*	1	*s*
16	2	12	6	11	6	*2*	...	...
17	3	11	2	8	4	*3*	...	L ...
18	3	11	6	8	4	*3*	...	...
19	3	12	2	11	6	*2*	...	...
20	3	12	4	8	4	*1*	...	...
21	3	12	4	11	6	*1*	...	...
22	3	12	6	8	4	*1*	...	...
				Heterozygous (1 pair)				
23	2	12	2·4	8	4	*2*	2	$2t^1$
24	2	12	2·4	11	4	*1*	...	...
25	2	12	2·6	11	6	*1*	...	...
26	2	12	2	11	4·6	*1*	...	...
27	3	12	2	11	4·6	*1*	...	...
				Homozygous (3x)				
28	2	12	2	11	7	*1*	...	...
					Total	61	9	

American species, *T. undulatum*. Small pieces of euchromatin intercalated within the non-spiralised H-segments have enabled the Japanese workers to describe a large number of forms of each chromosome (*e.g.* 23 A's in *T. kamtschaticum*).

TABLE 3

T. cernuum (*45 plants : 22 clones*)

Clone no.	Chromosome type and variant					No. of plants		No. and type of *f*
	A	B	C	D	E	No *f*	With *f*	
1	2	11	4	11	4	*3*	...	...
2	2	11	4	11	6	*1*	...	H
3	2	11	5	11	4	*1*	...	...
4	2	13	4	11	4	*1*	...	...
5	2	13	5	11	4	*1*	...	...
6	3	11	4	11	4	*6*	...	...
7	3	11	4	11	6	*2*	...	...
8	3	11	5	11	4	*5*	...	...
9	3	13	2	11	4	*1*	...	L
10	3	13	4	11	4	*5*	...	...
11	3	13	4	11	6	*1*	...	...
12	3	13	5	10	4	*1*	...	...
13	3	13	5	11	4	*6*	...	...
Heterozygous (1 pair)								
14	3	11·13	5	11	4	*1*	...	...
15	3	13	2·4	11	4	...	2	2*l*
16	3	11	4	10·11	6	*1*	...	...
17	2	11	5	10	4·6	*1*	...	...
18	3	11	2	11	4·6	*1*	...	...
19	3	11	4	11	4·6	*1*	...	...
20	3	11	4	11	4·6	*2*	...	...
21	3	13	2	11	4·6	*1*	...	...
22	3	13	5	11	4·6	*1*	...	...
					Total	43	2	

5. VARIATION IN H-SEGMENTS

(i) *Species*

The five basic chromosomes of *Trillium* are fairly distinct throughout the American species in their form combined with their pattern of H-segments (figs. 1-8). This pattern varies between species. But, in all our samples that include more than three plants, it also varies within species. This variation appears almost entirely as variation in the number, position and size of the H-segments.

In our whole collection the proportion of heterochromatin varies from 0·5 to 25 per cent. of the chromosome length. Within each species the range is smaller (table 11). Euchromatin may also vary as we see in the B1, B2, B3-series where the small blocks expose the

TABLE 4

T. luteum (*30 plants : 28 clones*)

Clone No.	Chromosome type and variant					No. of plants		No. and type of *f*
	A	B	C	D	E	No *f*	With *f*	
Homozygous								
1	1	8	2	4	1	...	3	*t*
2	1	8	2	5	2	...	1	*t*
3	1	9	2	5	4	...	1	s^1
4	1	10	2	5	1	1	...	...
Heterozygous for 1 pair								
5	1	6·8	2	4	1	1	...	... L
6	1	6·9	2	5	1	1	...	...
7	1	6·10	3	5	1	1	...	...
8	1	7·8	2	4	3	1	...	...
9	1	7·9	2	5	1	1	...	...
10	1	8·9	2	5	1	...	1	*s*
11	1	8·9	3	5	2	...	1	*s*
12	1	8·10	2	4	2	...	1	2*t*
13	1	8·10	2	5	2	...	1	*t*
14	1	9·10	4	5	3	1	...	... H
15	1	8	2·4	5	1	...	1	2*s*
16	1	8	2·4	5	1	...	1	*t*
17	1	9	2·3	4	1	...	1	*t*
18	1	10	2·4	5	2	1	...	...
19	1	8	2	2·4	3	...	1	*s*
20	1	7	2	4	1·3	...	1	*s*
21	1	8	3	5	2·3	1	...	...
22	1	10	2	5	1·2	1	...	...
Heterozygous for 2 pairs								
23	1	7·8	2·4	4	2	...	1	*t*
24	1	7·9	2·4	5	3	1	...	...
25	1	8·9	2	5	2·3	...	1	*s*
26	1	8	3	4·5	1·4	1	...	...
Heterozygous for 3 pairs								
27	1	6·8	2·4	4	1·3	1	...	...
28	1	8·9	2·3	4	2·3	1	...	...
					Total	14	16	

variation to our view. But it is obviously less variable in quantity than the heterochromatin.

Now the variation in the H-segments follows a similar pattern in different species: the polymorphism is parallel not only in each

TABLE 5

T. sessile (*20 plants: 13 clones*)

Clone no.	Chromosome type and variant					No. of plants		No. and type of *f*
	A	B	C	D	E	No *f*	With *f*	
				Homozygous				
1	1	9	1	4	1	*1*	...	...
2	1	9	■	4	1	*1*	4	*s*, 3*s*
3	1	10	2	4	1	...	1	*s*, H
				Heterozygous for 1 pair				
4	1	6·9	■	4	1	...	1	*s* L
5	1	7·9	1	4	1	*1*	1	*s*
6	1	7·9	■	4	1	...	1	*s*
7	1	7·10	2	4	1	...	1	*s*
8	1	9·10	1	4	1	*1*	...	...
9	1	9·10	■	4	1	*1*	■	*s*
10	1	9	1	4·5	1	*1*	...	...
11	1	9	2	4·5	1	...	1	2*s*
				Heterozygous for 2 pairs				
12	1	7·9	2	4·5	1	...	1	*t*
12	1	7·9	2	4	1·2	...	1	2*s*
					Total	6	14	

chromosome but in each arm of a chromosome. For example, the two arms of A are always similar, those of B always widely different. Each chromosome has its characteristic range and also its occasional unique variant. This parallelism would, no doubt, be more striking if more plants and more populations were studied.

How does this parallelism come about? It could have arisen in three ways:

First, free recombination might be occurring between species whose supposed differences were no barrier to crossing. This, however, seems to be excluded in *Trillium* by clear separation of many of our species. The results of species crossing are known only in polyploids found by Haga (1956) in Japan, but more hybridisation should be attempted.

TABLE 6

T. grandiflorum (*65 plants : 31 clones*)

Clone no.	Chromosome type and variant					No. of plants		No. and type of *f*
	A	B	C	D	E	No *f*	With *f*	
				Homozygous				
1	1	7	2	4	4	*2*	...	... L
2	1	7	2	5	4	...	1	*s*
3	1	8	1	4	4	...	1	*s*
4	1	8	2	4	2	4	1	*t*
5	1	8	2	4	4	11	5	*s*
6	1	8	2	5	4	*3*	1	*s*
7	1	8	7	4	4	*1*	1	*t*
8	1	9	2	4	2	*1*	...	...
9	1	9	2	4	4	*3*	2	*s*, 2*s*
10	1	9	7	4	2	*1*	...	...
11	1	9	7	4	4	*2*	...	...
				Heterozygous for 1 pair				
12	1	7·8	2	4	4	*1*	...	...
13	1	7·8	2	5	4	...	1	*s*
14	1	7·9	2	4	4	*1*	...	...
15	1	8·9	2	4	3	*1*	...	...
16	1	8·9	5	5	4	*1*	...	...
17	1	8·9	7	4	4	...	1	*t*
18	1	9	2·7	4	4	*3*	...	...
19	1	8	2	4·5	4	*2*	...	...
20	1	8	7	4	2·4	*2*	...	...
21	1	9	1	4	2·4	*1*	...	... H
22	1	9	2	4	2·4	...	2	2*s*
23	1	7	7	5	4·6	*1*	...	...
24	1	9	2	4	4·6	*1*	...	...
				Heterozygous for 2 pairs				
25	1	8·9	2	4	2·4	*1*	...	...
26	1	8·9	2	4	4·6	*1*	...	...
*27**	1	9	2·8	4·6	4	*1*	...	...
28	1	9	4·7	4·6	4	...	1	*t*
29	1	8	2	4·5	2·3	*1*	...	...
				Heterozygous for 3 pairs				
30	1	7·9	1·2	4	2·4	*1*	...	...
31	1	9	2·7	4·6	4·5	...	1	*t*
					Total	47	18	

* Plant *T.g.* 27 presents us with a problem in the distinction of C and D. It is here assumed to be heterozygous for the type C8. This plant is, however, also heterozygous for D6. We are now inclined to suppose that both these chromosomes are of the unique type C8 and that the C2 is a new type of D which we might number D12. The formula of the plant would then be C8.8, D4.12.

TABLE 7

T. stylosum (*20 plants : 10 clones*)

Clone no.	Chromosome type and variant					No. of plants		No. and type of *f*
	A	B	C	D	E	No *f*	With *f*	
				Homozygous				
1	1	4	2	2	1	*1*	...	...
2	1	5	1	3	2	*1*	...	...
3	1	5	1	2	1	*2*	...	...
4	1	5	1	2	2	*2*	...	...
5	1	5	2	2	2	*5*	...	...
6	1	5	2	1	2	*1*	...	...
7	1	5	2	2	1	*1*	...	...
8	1	5	2	3	2	*5*	...	...
9	1	6	1	3	1	*1*	...	...
				Heterozygous for 1 pair				
10	1	5	2·4	3	2	*1*	...	H
					Total	20	...	

TABLE 8

T. undulatum (*20 plants : 3 clones*)

				Homozygous				
1	1	1	1	7	4	*3*	...	...
2	1	2	1	7	4	*4*	...	H
3	1	3	1	7	4	*3*	...	L
					Total	10	...	

TABLE 9

(P. polyphylla (*9 plants : 3 clones*)

				Heterozygous				
*1**	1	2	1·2	1	1	...	1	*5*
2	1	1·2	2	2	2	*4*	1	*5*
3	1	1·3	2	3	3	...	3	*5*
					Total	4	5	

* Plant described by Darlington and La Cour (1938).

Secondly, pre-determining conditions might have led to parallel development of H-segments after the separation of the species. There

TABLE 10

Diagram of the general variation in chromosome complements of ten species of Trillium *showing parallel polymorphism. Each chromosome type is numbered. The numbers in the table are those of clone-chromosomes from tables 1-9. The numbers in squares are for types peculiar to one species. All others are common to several species.*

	A	B	C
T. grandiflorum	62	10 23 29 4 6 24 12 10 1 5 15 5	5 38 38 [10] 8 18	1 2 15 [1] 8
T. luteum	56		
T. sessile	26		
T. erectum	42 14	18 [38] 23 [21]	27 7	10 [19] 21 16
T. cernuum	12 32		
T. stylosum	20	[2] [16] 2	8 11 6	1
T. undulatum	6	[2] [2] [2]		
T. recurvatum	2	2	2	
T. ovatum	2	1 [1]		2
T. chloropetalum	4	4		4

are, of course, conditions limiting the development of H-segments. For example, although of the eighty-eight ends of the forty-four

TABLE 10—*continued*

chromosome variants twenty were heterochromatic only one variant had H-segments at both ends. There are, therefore, principles limiting

TABLE 10—*continued*

	D				E				f			
	1 2 3 4 5 6 7 8 9 10 11				1 2 3 4 5 6 7 8							
T. grandiflorum	47	12	[3]		12	3	43	[1] 3	7	6		
	20	36			24	16	13	3		5	9	
T. luteum	23	3			25	1			1	9		
T. sessile												
T. erectum	24						34	20 [2]	2	2	3	
		32									1	
T. cernuum		[7] 37					30	14				
T. stylosum	[2] [10] [8]				8	12						
T. undulatum			[6]				[6]					
T. recurvatum	2					2						
T. ovatum			[2] *		1	1						
T. chloropetalum			[4]		2			[2]			(1)	

the distribution of heterochromatin and probably equalising its distribution within and between the chromosomes perhaps to meet

the mechanical needs of mitosis or the physiological needs of the resting nucleus.

TABLE 11

Variation and heterozygosity among different chromosomes in species with the larger samples, indicating the range of heterochromatin content in the chilled chromosomes (L to H)

Species	No variants	With variants		Proportion of heterochromatin (per cent.)	
		No heterozygotes	Heterozygotes	L	H
T. erectum	...	A B ... D ...	... ... C ... E	16	19
T. cernuum	...	A	... B C D E	12	15
T. grandiflorum	A	... ...	... B C D E	6	10
T. luteum	A	... ...	... B C D E	3	9
T. sessile	A	... ... C ... E	... B ... D ...	5	8
T. stylosum	A	... B C D E	... ...	0·5	2

These general conditions, however, can hardly have decided the exact repetitions of pattern in different species. We prefer, therefore, the third possibility that the H-segments vary in parallel on account

TABLE 12

Frequency of structurally heterozygous pairs in relation to the number of variants of the five chromosome types in each species of Trillium *and* Paris

Species	No. of clones	A	B	C	D	E	Total	Heterozygous pairs (per cent.)	No. of variants*
T. grandiflorum	*31*	...	9	5	5	10	29	*18·7*	13
T. luteum	*28*	...	15	8	2	7	32	*22·8*	10
T. sessile	*13*	...	8	...	3	1	12	*17*	6
T. cernuum	*22*	...	1	1	1	6	9	*8·2*	6
T. erectum	*28*	...	...	3	...	2	5	*3·6*	7
T. stylosum	*10*	...	...	1	...	...	1	*2*	7
T. ovatum	*1*	...	1	...	...	1	2	...	2
P. polyphylla†	*4*	...	...	3	...	...	3	*15*	4
Total	*138*	...	34	21	11	27	93	...	...

* Variants are the numbers of types in excess of one for each of the five chromosomes.
† Includes one clone previously described.

of common descent from a similarly polymorphic ancestor whose variation has been preserved in the ten species. Thus we infer a very ancient and stable polymorphism depending on a conservation of the linear structure of the chromosome in respect of heterochromatin, or at least of intercalary heterochromatin.

This conclusion is in keeping with the general conservatism seen in the basic set of five chromosomes of *Trillium*.

(ii) *Populations*

The populations being variable we naturally wish to find out the frequency of heterozygotes for the variant chromosome types. The species-samples (or populations) with the most variants are of course,

TABLE 13

Frequencies of different types of particular chromosomes in combinations of pairs of homologues *

(i) *Trillium erectum* and *T. cernuum*

Difference in frequency of heterozygotes between A and E chromosomes.

	A			E		
	2·2	2·3	3·3	4·4	4·6	6·6
T. erectum	21	0	7	16	2	9
T. cernuum	6	0	16	12	6	4

(ii) *Trillium luteum* : B, 5 types

(56 chromosomes of 28 clones : *cf.* table 4)

II	6	7	8	9	10
6	... (0·15)	...	2	1	1
7		1 (0·32)	■	■	...
8			7 (5·1)	4	2
9				2 (1·25)	1
10					3 (0·90)
Total	1	6	24	12	10

Total { homozygotes *observed* : 13, *expected* : 7·7.
heterozygotes *observed* : 15, *expected* : 20·3.

* In table 13 the chromosome types are put in ascending order of H-content, *i.e.* the highest values are reached in B10, C8 and E5. The modal frequency is always at an intermediate content.

in general, those with the largest proportion of heterozygotes (table 12). Heterogeneity is a condition of heterozygosity—a principle Haga and Kurabayashi (1954) seem to have overlooked.

This frequency, it seems, is rarely equal to, and usually lower than the expectation with random recombination. But the deficiency of heterozygotes seems to differ for different chromosomes in the same plants from one population (table 13 (i)).

Taking the populations with the highest frequency of heterozygotes we have compared our observations with expectation on an assumption of random recombination and unselected survival (table

13 (ii)-(iv)). The results show that all possible combinations between and within chromosomes may well occur, as they should do in a freely

TABLE 13—*continued*

(iii) *Trillium grandiflorum* : C, 6 types
(62 chromosomes of 31 clones : *cf.* table 6)

C	*1*	*5*	*2*	*4*	*7*	*8*
1	■ (*0·2*)	…	1	…	…	…
5		1 (*0·032*)	…	…	…	…
2			17 (*11·7*)	…	2	1
4				… (*0·008*)	1	…
7					6 (*1·8*)	…
8						… (*0·008*)
Total	5	2	38	1	15	1

Total { homozygotes *observed* : 26, *expected* : 13·7.
heterozygotes *observed* : 36, *expected* : 48·3.

Note.—C2 is the commonest chromosome type as measured by our clones. Since it is two of our homozygous C2.2 clones that are represented by the largest numbers of plants (16 and 5) the clones may in fact be composed of identical seedlings.

(iv) *Trillium grandiflorum* : E, 5 types
(62 chromosomes of 31 clones)

E	*1*	*3*	*2*	*4*	*5*
1	3 (*1·2*)	5	1	…	…
3		17 (*15·0*)	…	1	3
2			1 (*0·07*)	…	…
4				… (*0·008*)	…
5					… (*0·07*)
Total	12	43	3	1	3

Total { homozygotes *observed* : 21, *expected* : 16·4.
heterozygotes *observed* : 41, *expected* : 45·6.

interbreeding population. But *within* chromosomes, homozygotes are again too numerous : in one sample only (E of *T. grandiflorum*) is there no serious deficiency of heterozygotes. Even here there would be serious deficiency if we admitted, what is most likely, that our multiple clone with sixteen plants (No. 5) was, in fact, sixteen seedlings.

The lack of apparent advantage of heterozygotes implies that even the heterozygosity of euchromatin adjoining the H-segments is not

TABLE 14

Combinations of chromosomes which are assumed to recombine two differences by crossing over in populations with different frequencies of heterozygotes in respect of the relevant chromosomes, compared with random expectation.

(i) *T. cernuum :* 22 clones, table 3

C	*1*	*4*	*2*	*5*	T
ab *1*	...	...	...	...	
AB *4*		10	*1*	...	
aB *2*			3	...	
Ab *5*				8	
Total	...	21	7	16	44
Expected	2·5	23·5	4·5	13·5	

(ii) *T. grandiflorum :* 31 clones, tables 6, 13 (iii)

C	*1*	*4+8*	*2+7*	*5*	T
ab *1*	2	...	1	...	
AB *4+8*		...	2	...	
aB *2+7*			25	...	
Ab *5*				1	
Total	5	2	53	2	62
Expected	6·55	3·55	51·45	0·45	

being favoured by selection. This situation is not unknown but it is in contrast to a common experience of inversion, interchange and fragmentation heterozygotes where euchromatin alone is in question.

The tables of Haga for his Sizunai population of *T. kamtschaticum* have been treated in the same way by our colleague, Mr A. F. Dyer (unpublished). He finds that homozygotes are in excess where larger numbers of chromosome types are present (in A, C and D). But in B and E, where there are few chromosome types and one or two are preponderant, the random heterozygote frequency is reached.

There is, however, a second kind of test that we can apply to these populations.

Intercalary H-segments can be recombined by crossing over as Rutishauser (1956*b*) has shown from the endosperm of *Trillium grandiflorum*. In three species we find pairs of chromosomes which differ in respect of the presence and absence of pairs of H-segments, giving four types of chromosome (table 14).

TABLE 14—*continued*

(iii) *T. luteum* : 28 clones, table 4

E	*1*	*4*	*2*	*3*	T
ab *1*	10	1	1	2	
AB *4*		1	...	...	
Ab *2*			6	3	
aB *3*				4	
Total	24	3	16	13	56
Expected	26·4	5·4	13·6	10·6	

(iv) *T. grandiflorum* : 31 clones, table 6

D	*4*	*5*	*6*	*Ab*	T
ab *4*	21	2	3		
aB *5*		5	...		
AB *6*			...		
Total	47	12	3	...	62
Expected	44·7	14·3	0·72	2·3	

Thus in *T. cernuum* and *T. grandiflorum* with parallel variation in C we have the situation in which we should expect that $1 \times 4 \rightleftharpoons 2 \times 5$. (In *T. grandiflorum* types 2 and 4 are also carried by other variants in respect of the other arm of the chromosome which can therefore be added to them.) The same situation can be used in E of *T. luteum* where $E2 \times 3 \rightleftharpoons E1 \times 4$. In each case we can represent the presence of an H-segment by *A* or *B* and its absence by *a* or *b* (fig. 4 and table 14).

These recordings show that the higher frequencies of heterozygotes in *T. luteum* give rise, as we should expect, to more nearly random recombination. The lower frequencies in the other species give disturbed ratios indicating selective elimination of some types (table 14). Especially notable is the disappearance of the non-H

type in *T. grandiflorum* at the same time that other high-H types have appeared.

Contrasted with the situation in *T. luteum* is that in the three clones of *Paris polyphylla* (table 9). Here D and E chromosomes both vary exactly as in the E of *T. luteum* giving the three types ab, aB and AB and they vary completely in parallel ; they are also all homozygous. Thus for the two pairs of chromosomes we have the formulæ :—

1. $\left\{D\ \frac{ab}{ab}\ \ E\ \frac{ab}{ab}\right\}$ 2. $\left\{D\ \frac{aB}{aB}\ \ E\ \frac{aB}{aB}\right\}$ 3. $\left\{D\ \frac{AB}{AB}\ \ E\ \frac{AB}{AB}\right\}$

This is the only example we have discovered of parallel variation between non-homologues within the complement. We therefore hope to obtain more specimens of this rare species.

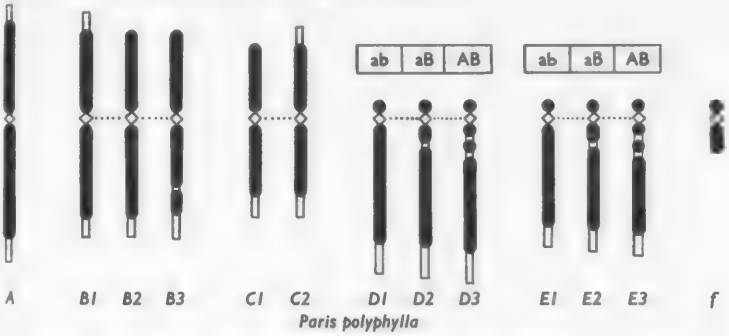


FIG. 7.—Complement diagram for the three clones of *Paris polyphylla*. × *c.* 1200.

6. EVOLUTION OF H-SEGMENTS

Comparative. We now have consistent evidence from three genera of flowering plants, and from *Triton* (Callan, 1942), of large chromosomes with blocks of heterochromatin capable of cold-starvation.

In each plant genus, *Paris*, *Trillium* and *Fritillaria*, there are species with and without heterochromatin. In each genus, also, the H-segments have a characteristic pattern of distribution. In *Fritillaria* (of California) and *Trillium* (of Japan) it is proximal ; in *Trillium* (of America) proximal in some chromosomes, and distal in others ; in *Paris polyphylla* it is chiefly distal. Further, the sizes of the H-segments are characteristic of the species. Where the H-contents are lowest they lie in very short proximal segments as in Japanese *Trillium* and in *Triton*. Where they are highest they lie in long terminal segments.

Earlier studies have shown the lack of connection of H-segments with crossing over in their distribution (Darlington, 1936, 1941). In *Trillium kamtschaticum* H-segments and crossing over are both proximally localised (Haga, 1952). In *Paris* H-segments are distal with crossing over proximal. And in *Fritillaria* H-segments are proximal and crossing over unlocalised.

The reason for this independence is no doubt that heterochromatin is condensed at the pachytene stage and from our evidence does not recombine by crossing over within its segments with euchromatin.

Where then do the H-segments come from? The earlier view was based on a comparison between species and on the supposed relations of sex chromosomes in *Drosophila*. It was that H-segments were allelic to euchromatin and the difference between the presence and absence of H-segments in different species was due to a difference in genotypic control acting on the same genes (Darlington, 1941). But the comparison of variants within the plant species now shows that this transformation does not apply here (table 10).

Both in Rutishauser's diagrams (1956*b*, fig. 6) and in our measurements, the H-segments (especially in the B's of *T. luteum* with terminal heterochromatin) appear to be additional when two homologues are compared. The simplest view is thus that they are replications of intercalated genes arising as a characteristic structural change in condensed

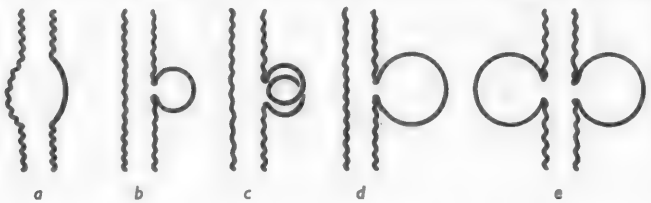


FIG. 8.—Pachytene diagram showing alternative relations of eu- and hetero-chromatin. (Wavy and straight lines) *a*, relations assumed in sex chromosomes of some animals, *b-e*, relations assumed in *Trillium* and other plants; *b*, original intercalation; *c*, first duplication by illegitimate crossing over; *d*, result of *c* : heterozygote; *e*, homozygote with non-pairing of heterochromatin.

segments (fig. 8). Such replications could preserve their individuality from the effects both of crossing over (which we know occurs in the adjacent euchromatin) and of other forms of breakage and reunion at pachytene. At pachytene H-segments in *Fritillaria pudica* seem to be condensed and incapable of pairing even when matched.

The evolutionary course with a replication of the H-segments can be seen in all stages in *Trillium* and in each stage in some other group of flowering plants, as follows (fig. 9) :—

(i) H-segments arise as single units or genes. They become recognisable when they are replicated to form small blocks (as in *Vicia faba*). They are then often localised near the centromere (*Trillium kamtschaticum*).

(ii) Larger blocks are formed by further replication and give several blocks in each chromosome arm and in different chromosomes by translocation. The blocks are thus supernumerary, internally repetitive but freed from internal crossing over by pachytene condensation (*Fritillaria pudica* and *T. stylosum*).

(iii) By crossing over these blocks are recombined. Some of the products of recombination, like B9, B11, B13 and B14, no longer occur in the same species. Their relics in different species show the consequences of crossing over and selective survival at an earlier stage of their history.

(iv) Where even larger blocks are required this is sometimes possible in a proximal position as in the California species of *Fritillaria* (La Cour, 1951). To produce this result intercalary H-segments can

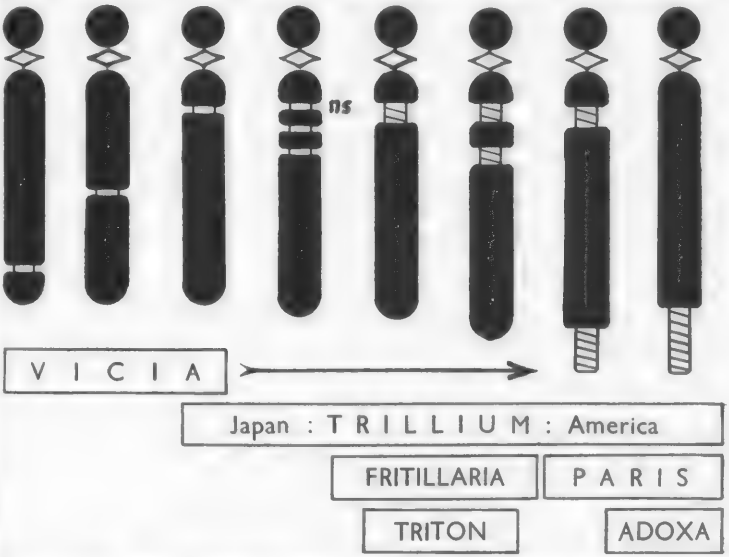


FIG. 9.—Eight types of H-segment pattern found in single chromosome arms of six genera to show that evidence of an evolutionary sequence, represented by the arrow, may be derived from *Trillium*. *ns*, short segment capable of non-spiralising : only one per arm and only with the long arm distal to it.

Sources.—Vicia, McLeish, 1952 ; *Trillium*, Haga, 1956 ; *Fritillaria*, Darlington and La Cour, 1941 ; *Paris*, Darlington and La Cour, 1938 ; *Triton*, Callan, 1942 ; *Adoxa*, Geitler, 1940.

increase in size as shown by C3 in *T. luteum* and D5′ in *T. ovatum* ; perhaps also in *Lachenalia* (Therman, 1957).

(v) This faculty is not usually exploited perhaps because a long intercalary H-segment is liable to obstructed division when starved. It will then stick at anaphase and so imperil the euchromatin distal to it (Darlington and La Cour, 1940, Shaw, 1958). Translocation towards the ends will thus usually be favoured. Hence will arise, first, the B1 or B12 pattern, and later the terminal position which is established in B and D in most American species of *Trillium* and is beginning in C. Owing to their liability when starved to form bridges which break unequally these end segments can be rapidly extended. The extensions will again always be repetitive.

(vi) Thus the species of *Trillium* (*T. chloropetalum* and *T. recurvatum*) with the highest H-content represent the nearly final stage in this evolution. Similarly the terminal position for the H-segments has almost displaced the intercalary in *Paris polyphylla* and altogether displaced it in *Adoxa moschatellina* (Geitler, 1940). This last stage is compatible with a very high H-content. It is therefore most prominent and was the first to be observed.

In the evolution of *Trillium*, as we see, H-segments seem to jump from proximal to distal positions. Why? In species growing where temperatures are likely to lead to DNA starvation the mechanical consequences of this starvation will no doubt affect the distribution of H-segments. The activities of the H-segments in the resting nucleus, where (by contrast with nucleoli) they seem to need contact with the nuclear membrane, may however also affect their distribution (*cf.* Therman, 1957). Probably for this reason H-segments so rarely lie in the middle of long arms or at the end of short arms.

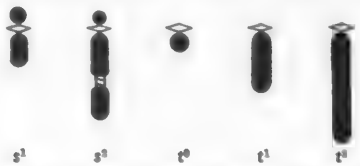


FIG. 10.—The five types of supernumerary found in *Trillium* and *Paris* with their symbols: sub-telocentric, s; telocentric, t. s^1-2·5 μ, s^2-9 μ, t^0-1·5 μ, t^1-4·0 μ, t^2-10 μ. × *c.* 1800.

7. SUPERNUMERARY CHROMOSOMES

In addition to the five pairs of what we may call autosomes, sixty-one plants had supernumerary chromosomes. These we refer to as f rather than B in *Trillium*. They have not been found in the Japanese species.

Constancy. In tables 2-9 the total numbers of plants in each clone are given, the clones being defined by the uniformity of H-segment pattern in the several plants of each. Within rhizomes (even four branching rhizomes of *T. erectum*) the numbers of supernumeraries have proved to be constant. Evidently, therefore, somatic change, if it occurs, is less frequent than elsewhere (*cf.* Håkansson, 1956, on *Godetia*; Bosemark, 1957, on *Poa trivialis*).

In those clones which include several plants, however, the plants may vary in number of supernumeraries. Thus, of our 138 "clones", forty-seven had supernumeraries. Of these, seventeen, or nearly all of those which included several plants, varied in the number of supernumeraries. We shall refer to the groups with constant supernumeraries as *sub-clones*.

How do these sub-clones arise? In *T. erectum*, *T. sessile*, *T. grandiflorum* and *P. polyphylla* there are altogether ten clones with none and

with one supernumerary. In these cases we believe we may be concerned with genuine sub-clones derived by loss of the supernumerary at mitosis. There are, however, discontinuous types of sub-clone in *T. erectum* (clones 11, 14 and 23) and *T. sessile* (clone 1). In the extreme case two sub-clones had none and six fragments. Such sub-clones must be really different clones from different seedlings.

TABLE 15

Numbers of clones with different numbers of supernumeraries in Trillium *and* Paris (*128 clones, 35s, 35t*)

Species	None	*s*	*t*	Type : μ	Number					T	M
					1	2	3	4	6		
T. erectum . .	21	2	5	s^1 : 2·5	2	...	...	...	...	2	0·6
28 clones				t^0 : 1·5	...	...	...	1*	1*	10	(1·4 μ)
				t^1 : 4·0	1	2†	...	...	...	5	
T. cernuum . .	21	...	1	t^1 : 4·0	...	1	...	...	...	2	0·1
22 clones											(0·2 μ)
T. luteum . .	14	5	9	s^1 : 1·5	4	1	...	...	...	6	0·6
28 clones				t^2 : 10·0	7	2	...	...	...	11	(4·1 μ)
T. sessile . .	3	9	1	s^1 : 2·5	6	2	1‡	...	...	13	1·1
13 clones				t^0 : 1·5	1	...	...	...	...	1	(2·6 μ)
T. grandiflorum .	19	8	6	t^1 : 2·5	6	2	...	...	...	10	0·5
33 clones ‡				t^2 : 4·0	6	...	...	...	...	6	(1·5 μ)
T. ovatum . .	...	1	...	s^2 : 9·0	1	...	...	...	...	1	1·0
1 clone											
P. polyphylla . .	...	3	...	s^1 : 2·5	3	...	...	...	...	3	1·0
3 clones											

* Sub-clones had no *ff*.

† Sub-clones had 1 and 0 *ff*.

‡ Three sub-clones in terms of autosomes were of the three different supernumerary types and are counted as 3 clones here.

Types. The supernumeraries of *Trillium* and *Paris* are of two main types, sub-telocentric (*s*) and telocentrics (*t*). There is never more than one type within an individual and there are no iso-chromosomes. The centromeres are thus fairly stable and both types may be in their original form, *s* being derived by breakage of heterochromatin and *t* by misdivision of the centromere (table 15).

All the supernumeraries save one in *T. ovatum* are entirely euchromatic. There are five sizes of supernumerary from 1·5 μ to 10 μ in length. The 10 μ and 4 μ types never exceed two in number; the 2·5 μ type three, and only the 1·5 μ type reaches six. Indeed they represent a source of variation, reaching 10 per cent. in *T. luteum*, of the length of euchromatin which, otherwise, within the autosomes, is almost invariable. As a fraction of the whole complement this

corresponds to the variation in heterochromatin afforded by the H-segments.

The supernumeraries in grasses (*Zea*, *Secale*, *Sorghum*, *Anthoxanthum*, *Festuca and Poa*) are heterochromatic or largely so (*cf.* Bosemark, 1957). In these grasses, however, there is little heterochromatin in the autosomes. In maize, indeed, heterochromatin seems to have been developed in the autosomes of improved varieties to replace what has been lost in the supernumeraries (Darlington and Upcott, 1941).

Boosting Mechanisms. In plants we know four mechanisms which conduce to the maintenance or increase of supernumeraries and compensating for their loss of meiosis.

(i) Pairing and spiralisation of supernumeraries lapses at first metaphase of meiosis and the univalents pass to one pole (*Parthenium :* Catcheside, 1950).

(ii) Both chromatids of supernumeraries pass to the generative nucleus at the first pollen mitosis (*Sorghum*, *Secale*, *Anthoxanthum*, *Festuca*, *Phleum*, etc. : Darlington and Thomas, 1941, Müntzing, 1946).

(iii) Pollen tubes with supernumeraries grow faster than those without them : hence they preferentially fertilise the eggs (*Zea*, Roman, 1948).

(iv) Supernumeraries arepre ferentially included at embryo-sac meiosis in the egg-cell nucleus (*Lilium*, Kayano, 1956 ; *Trillium*, Rutishauser, 1956*a*).

In *Trillium* the supernumeraries are normally distributed in the pollen mother cells with some elimination (Sparrow, 1952). In the embryo-sac they are boosted. It is significant that only heterochromatic supernumeraries can be directed in the pollen grain mitosis since the direction depends on sticking of H-segments at anaphase. Euchromatic supernumeraries depend on embryo-sac direction.

Evidently supernumeraries are keeping themselves going in populations of nearly all the American species of *Trillium*. The boosting mechanism compensates for loss at meiosis. Plants with and without supernumeraries are competing in nature on equal terms, as they are in so many hundreds of other plant genera consisting of herbaceous or annual diploid species.

Like all other forms of balanced polymorphism each alternative is advantageous in a part of the population.

8. ORIGINS OF POLYMORPHISM

(i) *Levels and modes*

Balanced polymorphism in the sense in which Ford (1957) has developed the notion depends on the maintenance by selection of an equilibrium between alternative or mutually replaceable genotypes in an interbreeding population. It differs from new or sporadic variation in that the two alternatives will have had a long time to

modify their own genetic neighbourhood by mutual selection, a process which will always encourage the development of a super-gene. Any development of this kind of discontinuity is an integrative and evolutionary process like that of species formation but on a reduced scale. Its study may rely on the phenotype, as when it concerns mimicry, caste-formation, blood antigens, breeding systems and cytoplasmic polymorphisms. Or it may rely on the chromosomes as when it concerns inversions, interchanges, fragmentation, supernumerary chromosomes, iso-chromosomes and H-segments. Or again, it may combine both methods as we see in sex determination, the many-tiered sex-ratio system of *Drosophila*, or the complex-heterozygote in *Œnothera*.

The combination of the chromosome and phenotypic approaches to polymorphism shows us an interlocked system of interpretation whose parts need to be disentangled. Crossing over suppression and floating structural variation are opposite aspects of the genetic isolation of segments and the evolution of super-genes (*cf.* Darlington, 1956). The combination of the two methods and aspects illuminates the immense range of studies of polymorphism, by analogy, by antithesis and even by paradox.

Take first an analogy. All methods demonstrating parallel polymorphism are also means of tracing phylogeny. Thus with the hybrid species of *Œnothera* the variable interchanges show parallel polymorphism, while the constant and conservative pairing segments preserve the phylogeny throughout whole sections of the genus. Similarly, the sex-ratio inversion complex in *Drosophila* and the blood-group genes in men and monkeys demonstrate parallel polymorphism and reveal phylogeny over long ranges of descent. Parallel polymorphism in the H-segments of *Trillium*, therefore, although unusually striking, merely extends a principle already revealed in several other ways.

Secondly, consider an antithesis. New variations arising in the chromosomes, whether mutations or structural changes, encounter various kinds of situation in which they can spread. These situations may differ externally (Mather, 1955). They may also differ internally. On the one hand, there is that in which they benefit by *genetic isolation* from their alternatives at the diploid level. Such variations underlie the separation of species. On the other hand, there is that in which they benefit by *genetic combination* with their alternatives at the diploid level. This situation favours genetic isolation at the haploid level. It underlies polymorphism ; it underlies the development of super-genes and gene complexes ; and it underlies developments of H-segments and supernumeraries such as we have been considering.

Again, within this second group, we have an antithesis between those in which selection is indifferent as between heterozygote and homozygote and those in which it favours the heterozygote. Now polymorphisms which depend on the advantage of the heterozygote

may lead to the complex heterozygote in which no homozygotes exist (as first discussed by Bateson, 1902). The population of zygotes has then ceased to be polymorphic but the polymorphism may be said to have been transposed to the haploid level. This happens with both interchange and fragmentation polymorphisms, and with both together in *Campanula* (*cf.* Darlington, 1956*a* and *b*).

Comparison of these situations indicates that selection maintains or advances a polymorphism either by disfavouring certain homozygotes inherently or by disfavouring them only when they are excessively frequent, that is by encouraging differences as such. H-segments and supernumeraries prosper, as we have seen, by the second means : not by favouring heterozygotes, but by generating

TABLE 16

Contrasted properties of the two replicatory types of polymorphism

Property	H-segments	Supernumeraries
heterochromatic . .	always : and stable	usually : and unstable
euchromatic . . .	...	sometimes : and stable
internal crossing-over .	none	only with sisters
Results of meiosis :—		
(i) chromosomal . .	reduction leads to recombination	loss or doubling
(ii) populational and individual	thus heterogeneity leads to heterozygosity	thus heterogeneity does not lead to heterozygosity

diversity. They both provide a source of variation for the population : they pre-adapt it to the needs of a fluctuating environment. Although both of them are based on replication they achieve this end by contrasted means (table 16).

(ii) *Alternative types*

The contrast of properties between H-segments and supernumerary chromosomes is instructive in two respects. First, it shows that heterochromatin is always relatively unstable when it makes a whole chromosome : it is liable to misdivision and also fragmentation. But it is relatively stable when wedged between large segments of euchromatin. Secondly, it shows us that supernumeraries can be either heterochromatic or euchromatic.

Now, the established supernumeraries of *Trillium* and *Paris* are euchromatic. Are they then co-operating with the heterochromatic material in the autosomes ? Or are the two in some sense competing ? Can it be that the H-segments in providing variation within the autosomes in respect of heterochromatin have freed the supernumerary mechanism as a basis of variation in euchromatin ?

Answers to these questions can be obtained by comparing the occurrence of supernumeraries in species with different H-contents,

that is in diploid species, for established polyploids have neither. There is evidently a correlation (table 17). This correlation should apply to other species of *Trillium*. *T. ovatum*, *recurvatum* and *chloropetalum* should also prove to have euchromatic supernumeraries when large samples are studied.

This comparison makes it probable that the two sources of variation in heterochromatin and in euchromatin are co-operating and not competing in *Trillium*. It is not to be supposed that this is a general principle. Nor is it to be supposed that it answers the basic questions. We are inclined to suppose that the H-segments of *Trillium*, on the one

TABLE 17

Relation between presence of heterochromatin in autosomes and euchromatic supernumeraries in diploid species of Trillium *and* Paris

Variable euchromatin		Variable heterochromatin	
		Much : >5 per cent.	Little : <5 per cent.
	With supernumeraries	*Trillium* : 5 American species *Paris polyphylla*	none
	Without supernumeraries	none	*Trillium* : 3 Japanese species *T. stylosum*

hand, are replications of the same stuff and of great age. The supernumeraries, on the other hand, being euchromatic, are not replicated but are differentiated internally. In view of the great heterogeneity of *T. erectum* which had three types of supernumerary in twenty-eight clones, each no doubt qualitatively different from the others, we must also assume that they are constantly being replaced.

The great development of heterochromatin in *Trillium* is an indication of mechanically or physiologically unusual conditions. Its situation is nevertheless important as showing how different groups of organisms with similar mechanisms of heredity may have entirely different mechanisms of variation.

It shows us particularly that, at the chromosome level, we may contrast two kinds of polymorphism. Those which are phenotypically recognisable are *allelic*. Those which are not so recognisable are *replicatory*.* Pairing in haploids shows that a great deal of replicatory variation is going on under the surface in most organisms. But supernumerary chromosomes and H-segments are visibly replicatory. They both arise as replications and they both seem to proliferate by further replication. Their action is non-specific and incapable of

* It would be far-fetched to call them Morganian and Batesonian, but we may note that replication implies presence-and-absence.

heterosis. This we expect of heterochromatin. We may also expect it of any other replication beyond the diploid level such as that of supernumeraries.

The suggestion that the replicatory polymorphisms act merely by increasing variability in a quantitative sense presents us with an interesting contradiction. For such replicatory variation would seem likely to provide the basis of certain kinds of polygenic variation. Now polygenic variation has not been thought of as connected with polymorphism because its discontinuity is invisible except at the chromosome level. But so also is the contrast between the allelic and the replicatory situations.

The present studies continue a development which has proceeded unnoticed ever since the rediscovery of Mendelism. For throughout this time we have been increasingly impressed with the effect of chromosomes as determining heredity. This proper interest in *determining* heredity has distracted us, however, from their other property of *concealing* variation. Or, as we may put it, their property of carrying invisible but useful ballast.

Most new alleles, being recessives, most structural changes in chromosomes, most polygenic variation, most heterochromatin, most supernumerary chromosomes, and even a great deal of polyploidy : all these are concealed from us. Each is a cryptic variation. All of them combined give us cryptic species. Heredity we discover is a reservoir of concealed variation. And it is in this respect that genetics has most changed our view of the process of evolution from that which could be envisaged in the nineteenth century by Lamarck or by Darwin or indeed by anyone except Mendel.

9. SUMMARY

1. The heterochromatin in H-segments has been mapped by DNA-starvation of the chromosomes in samples of ten species of *Trillium* and one of *Paris* (table 1, figs. 1-8).

2. There are three degrees of starvation depending on differences in physiological conditions (*cf.* Shaw, 1958, 1959, Heyes and Shaw, 1958).

3. In the five pairs of autosomes (A-E) the 273 plants showed 142 types of chromosome complement differing in number and position of H-segments. Some complements found in several plants are assumed to be clonal replicates for the present purpose (table 10).

4. The proportion of heterochromatin varies in the group from 0·5 per cent. to 25 per cent. and over a range of 1 : 4 within species (table 11).

5. Of the ten supposed species of *Trillium* two pairs overlap in external form and in H-segment variation. There are thus eight genetic species. Of these, four in large samples from North Carolina showed wide internal variation.

6. Size, position and frequency relations between H-segments revealed five principles :—

(i) Homologous chromosomes within species vary in their H-segments mainly owing to addition to the euchromatin and not to conversion of it.

(ii) Homologous chromosomes vary in parallel within and between species. This indicates a parallel balanced polymorphism derived from common ancestors.

(iii) Whole complement comparisons indicate that the H-segments began in *Trillium* in intercalary positions near the centromere, the *Fritillaria-Vicia* pattern. Later they have been shifted to the ends to give the *Paris* pattern in one or two chromosomes.

(iv) Allelic relations show that heterozygosity is not favoured (table 13).

(v) Double-allelic relations show that crossing over is free in *T. erectum* if not in *P. polyphylla* (fig. 10, table 14).

7. In sixty-one plants of the seven species having most heterochromatin there were supernumerary chromosomes. All were of small standard types, subtelo- or telo-centric, and all, save one, were entirely euchromatic.

8. The correlation between supernumerary chromosomes and H-segments is not unexpected. They provide corresponding sources of variability in euchromatin and in heterochromatin. Both are most strongly developed in diploid species. Both afford a *replicatory* and non-specific, as opposed to an *allelic* and specific basis of polymorphism.

10. REFERENCES

BAILEY, L. H. 1941. *The Standard Cyclopedia of Horticulture.* Macmillan, New York.

BAILEY, P. C. 1949. Differential chromosome segments in *Trillium erectum* L. *Bull. Torrey Bot. Club, 76,* 319-336.

BAILEY, P. C. 1952. Differential reactivity in six species of *Trillium. Bull. Torrey Bot. Club, 79,* 451-458.

BAILEY, P. C. 1954. Differential reactivity of chromosomes as an indicator of species in *Trillium. Bot. Gaz., 115,* 241-248.

BATESON, W. 1902. The facts of heredity, etc. *Sci. Papers* (Cambridge, 1928), *2,* 37.

BOOTHROYD, E. R. 1953. The reaction of *Trillium* pollen tube chromosomes to cold treatment during mitosis. *J. Heredity, 44,* 3-9.

BOSEMARK, N. O. 1957. Further studies on accessory chromosomes in grasses. *Hereditas, 45,* 236-297.

BRITTON AND BROWN (AND GLEASON, H. A.). 1952. *Flora of the United States, etc.* New York.

CALLAN, H. G. 1942. Heterochromatin in *Triton. P.R.S.,* B, *130,* 324-335.

CATCHESIDE, D. G. 1950. The B-chromosomes of *Parthenium argentatum. Genet. Iber., 2,* 139-148.

DARLINGTON, C. D. 1936. The external mechanics of the chromosomes. *P.R.S.,* B, *121,* 264-319.

DARLINGTON, C. D. 1941. Polyploidy, crossing over and heterochromatin in *Pairs*. *Ann. Bot.*, *5*, 203-216.

DARLINGTON, C. D. 1956*a*. Natural populations and the breakdown of classical genetics. *P.R.S.*, B, *145*, 350-364.

DARLINGTON, C. D. 1956*b*. *Chromosome Botany*. Allen & Unwin, London.

DARLINGTON, C. D. 1957. Messages and movements in the cell. *Conference on Chromosomes*. Willink, Zwolle, Netherlands.

DARLINGTON, C. D., AND LA COUR, L. F. 1938. Differential reactivity of the chromosomes. *Ann. Bot.*, *2*, 615-625.

DARLINGTON, C. D., AND LA COUR, L. F. 1940. Nucleic acid starvation of chromosomes in *Trillium*. *J. Genet.*, *40*, 185-213.

DARLINGTON, C. D., AND LA COUR, L. F. 1941. The detection of inert genes. *J. Heredity*, *32*, 115-121.

DARLINGTON, C. D., AND LA COUR, L. F. 1945. Chromosome breakage and the nucleic acid cycle. *J. Genet.*, *46*, 108-267.

DARLINGTON, C. D., AND LA COUR, L. F. 1950. Hybridity selection in *Campanula*. *J. Heredity*, *4*, 217-248.

DARLINGTON, C. D., AND THOMAS, P. T. 1941. Morbid mitosis and the activity of inert chromosomes in *Sorghum*. *P.R.S.*, B, *130*, 122-150.

DARLINGTON, C. D., AND UPCOTT, M. B. 1941. The activity of inert chromosomes in *Zea mays*. *J. Genet.*, *41*, 275-296.

FORD, C. E., HAMERTON, J. L., AND SHARMAN, G. B. 1957. Chromosome polymorphism in the common shrew. *Nature*, *180*, 392-393.

FORD, E. B. 1957. Polymorphism in plants, animals and man. *Nature*, *160*, 1315-1319.

GEITLER, L. 1940. Temperaturbedingte Ausbildung von Spezialsegmenten au Chromosomsnenden. *Chromosoma*, *1*, 554-561.

GILES, R. A., AND WILSON, G. B. 1956. A cytological and morphological study of two populations of *Trillium grandiflorum*. *Cytologia*, *21*, 376-383.

GRAY (AND FERNALD, M. L.). 1950. *Manual of Botany*, New York.

HAGA, T. 1952. Failure of pairing in *Trillium kamtschaticum*. *Jap. J. Genet.*, *27*, 72-74.

HAGA, T. 1956. Genome and polyploidy in the genus *Trillium*. VI. Hybridisation and speciation by chromosome doubling in nature. *Heredity 10*, 85-98.

HAGA, T., AND KURABAYASHI, M. 1953. Genome and polyploidy in the genus *Trillium*. VI. Differential reaction of chromosome segments to low temperature. *Cytologia*, *18*, 13-28.

HAGA, T., AND KURABAYASHI, M. 1954. Genome and polyploidy in the genus *Trillium*. V. Natural populations of *kamtschaticum*. *Mem. Fac. Sci. Kyushu Univ.* (*E*) *Biol.*, *1*, 159-185.

HÅKANSSON, A. 1956. Spontaneous chromosome variation in the roots of a species hybrid. *Hereditas*, *32*, 39-59.

HEYES, J. K., AND SHAW, H. W. 1958. Chemical determination of Deoxyribonucleic acid in root tips before and after cold treatment. *Nature* 181, 1377-1388.

KAYANO, H. 1956. Cytogenetic studies in *Lilium callosum*. II. Preferential segregation of supernumerary chromosomes. *Mem. Fac. Sci. Kyushu Univ.* (*E*) *Biol.*, *2*, 53-60.

KURABAYASHI, M. 1957. Evolution and variation in *Trillium*. IV. Natural populations of *T. kamtschaticum*. *Jap. J. Bot.*, *16*, 1-45.

LA COUR, L. F. 1951. Heterochromatin and the organisation of nucleoli in plants. *Heredity*, *5*, 37-50.

MCLEISH, J. 1952. The action of maleic hydrazide in *Vicia*. *Heredity*, *6* (*Sup. Chr. Breakage*), 125-147.

MATHER, K. 1955. Polymorphism as an outcome of disruptive selection. *Evolution*, *9*, 52-61.

MÜNTZING, A. 1946. Cytological studies of extra fragment chromosomes in rye. III. The mechanism of non-disjunction at pollen mitosis. *Hereditas*, *32*, 97-119.

ROMAN, N. 1948. Directed fertilisation in maize. *P.N.A.S.*, *34*, 36-42.

RUTISHAUSER, A. 1956*a*. Genetics of fragment chromosomes in *T. grandiflorum*. *Heredity*, *10*, 195-204.

RUTISHAUSER, A. 1956*b*. Chromosome distribution and spontaneous chromosome breakage in *T. grandiflorum*. *Heredity*, *10*, 376-407.

RUTISHAUSER, A., AND LA COUR, L. F. 1956. Spontaneous chromosome breakage in hybrid endosperms. *Chromosoma*, *8*, 317-340.

SHAW, G. W. 1950. A rapid permanent method for chromosomes. *Nature*, *165*, 408.

SHAW, G. W. 1956. The preparation of chromosomes. *Microscope*, *11*, 43-45.

SHAW, G. W. 1958. Adhesion loci in the differentiated heterochromatin of *Trillium* species. *Chromosoma* 9; 292-304.

SHAW, G. W. 1959. The nature of differential reactivity in the heterochromatin of *Trillium* and *Paris* species. *Cytologia* (in the press).

SPARROW, A. H., *et al*. 1952. Distribution and behaviour of supernumerary chromosomes during microsporogenesis in a population of *T. erectum* L. *Amer. Nat.*, *86*, 277-292.

THERMAN, E. 1957. Chromocentres in the mitosis of *Lachenalia*. *Arch. Soc. Vanamo*, *11*, 189-193.

WALLACE, B. 1948. Studies on " Sex-Ratio " in *Drosophila pseudoobscura*. I. Selection and " Sex-Ratio ". *Evolution*, *3*, 189-217.

WILSON, G. B., AND BOOTHROYD, E. R. 1941. Studies in differential reactivity I. The rate and degree of differentiation in somatic chromosomes. *Canad. J. Res.*, *19*, 400-412.

WILSON, G. B., AND BOOTHROYD, E. R. 1944. Temperature-induced differential contraction in the somatic chromosomes of *Trillium erectum*. *Canad. J. Res.*, *22*, 105-119.

NOTES AND COMMENTS

THE 2×2 GENOTYPE-ENVIRONMENT TABLE

J. H. VAN DER VEEN

Laboratory of Genetics, Agricultural University, Wageningen, The Netherlands, and A.R.C. Unit of Biometrical Genetics, Department of Genetics, University of Birmingham, England

Received 4.ix.58.

If two genotypes or groups of genotypes, G_0 and G_1, are raised in each of two environments, E_0 and E_1, the four resulting phenotypes may, in respect of any quantitative character, be expressed in terms of four parameters. In table 1, m stands for the overall mean, d represents the average genotypic effect, e the average environmental effect, and g the genotype-environment interaction. These parameters were recently used by Mather and Morley Jones (1958) in their study of genotype-environment interactions in quantitative inheritance.

TABLE 1

	G_0	G_1	Mean
E_0	m+d+e+g	m−d+e−g	m+e
E_1	m+d−e−g	m−d−e+g	m−e
Mean	m+d	m−d	m

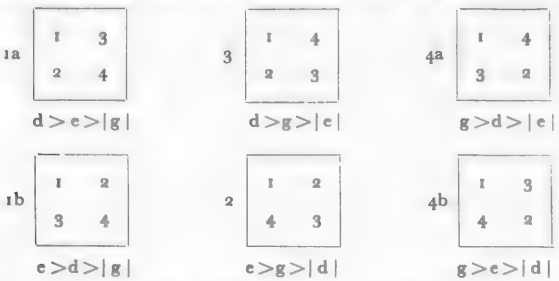
TABLE 2

	G_0		G_1	
E_0	00	d+e+g	01	e
E_1	10	d	11	g

In the following we will compare in terms of d, e and g the *relative* magnitudes of the phenotypic values, to be labelled 1, 2, 3 and 4 in descending order. For ease of inspection table 1 may be transformed into table 2 by adding to its elements −m+d+e+g and dividing by 2.

Haldane (1946) compared the relative magnitudes without the use of parameters, and dealt with $3! = 6$ situations, left after assigning the largest phenotypic value to 00. This implies (table 2) e+g>0, d+g>0, and d+e>0, *i.e.* not more than one of the parameters is allowed to become

negative, and its absolute value must be smaller than the value of the two positive ones. Haldane's six types can be described as follows :—



Mather and Morley Jones (*loc. cit.*) took all three parameters as positive in that they omitted the modulus sign from the inequalities, and therefore did not cover all possible situations.

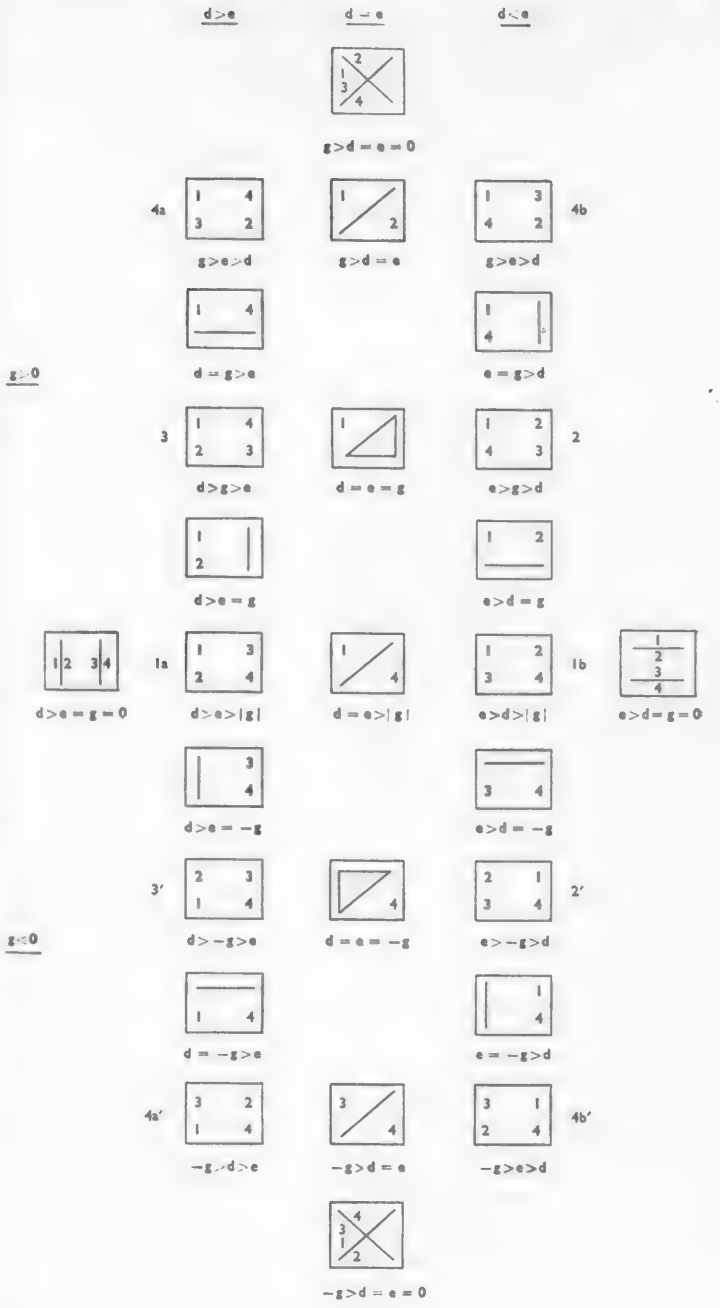
The present author proposes a modified approach as (1) it seems more consistent to take the same two parameters as positive in all cases, and as (2) it is customary in biometrical genetics to define d as non-negative. Let us define d and e as non-negative and consequently allow only g to take sign. This means that the four phenotypic values must be arranged in table 1 so that $00+10 \geqslant 01+11$, as $d \geqslant 0$, and $00+01 \geqslant 10+11$, as $e \geqslant 0$. Then with $g<0$ four new sequences of magnitude arise, *viz.* 3′ ($d>-g>e$) as a counterpart of 3 ($d>g>e$), etc., in which 00 is not the largest phenotype. The $6+4 = 10$ types have been listed in table 3. Inspecting this table with the help of table 2 we see :—

1. Table 3 is symmetrical for $d>e$, *i.e.* $10>01$, and $e>d$, *i.e.* $10<01$.
2. $g>0$ implies that 00 is the largest phenotype, $g<0$ that 11 is the smallest.
3. If $g>0$, then $00-10>|01-11|$ and $00-01>|10-11|$. This means that G_0 is the less stable genotype and at the same time E_0 the most differentiating environment. If $g<0$ this holds for G_1 and E_1 respectively.
4. If $d>|g|$ and $d>e$ (cases 1*a*, 3 and 3′), then G_0 is the highest "yielder" irrespective of environment : G_0 shows *unconditional superiority*. If in additon $|g|>e$ (cases 3 and 3′), then G_0 and G_1 have their highest values in different environments. Thus the superiority of the environment is conditioned by the genotype. This may be described as *conditional superiority of the environment*.

   If $e>|g|$ and $e>d$ (cases 1*b*, 2 and 2′), then E_0 shows *unconditional superiority*. If in addition $|g|>d$ (cases 2 and 2′), then

TABLE 3

The possible comparisons in terms of the parameters d, e and g (cf. *Table* 1), *of four phenotypes obtained by raising two genotypes in each of two environments. The phenotypic values have been labelled 1, 2, 3 and 4 in descending order of magnitude. The parameters d and e have been defined as non-negative. A line indicates equality of phenotypic values. Type numbers have been added to the cases where all four values are different.*


d>e
d = e
d<e
g>d = e = 0
4a
4b
g>e>d
g>d = e
g>e>d
d = g>e
e = g>d
g>0
3
2
d>g>e
d = e = g
e>g>d
d>e = g
e>d = g
d>e = g = 0
1a
1b
d>e>|g|
d = e>|g|
e>d>|g|
e>d = g = 0
d>e = −g
e>d = −g
3′
2′
g<0
d>−g>e
d = e = −g
e>−g>d
d = −g>e
e = −g>d
4a′
4b′
−g>d>e
−g>d = e
−g>e>d
−g>d = e = 0

E_0 and E_1 induce the " better performance " in different genotypes. Thus the superiority of the genotype is conditioned by the environment : *conditional superiority of the genotype.*

If $|g| > d$ and $|g| > e$ (cases 4*a*, 4*a'*, 4*b* and 4*b'*), then there is no unconditional superiority of either kind : *specific adaptation.*

Turning now to the cases of two or more phenotypic values being equal, we find that each of them corresponds uniquely to a parameter equality (*cf.* table 2) :—

$$d = -g \quad \therefore \; 00 = 01 \qquad e = -g \quad \therefore \; 00 = 10$$

$$d = g \quad \therefore \; 10 = 11 \qquad e = g \quad \therefore \; 01 = 11$$

$$d = g = 0 \quad \therefore \begin{cases} 00 = 01 \\ 10 = 11 \end{cases} \qquad e = g = 0 \quad \therefore \begin{cases} 00 = 10 \\ 01 = 11 \end{cases}$$

$$d = e \quad \therefore \; 10 = 01 \qquad d = e = -g \quad \therefore \; 00 = 01 = 10$$

$$d = e = 0 \quad \therefore \begin{cases} 10 = 01 \\ 00 = 11 \end{cases} \qquad \begin{matrix} d = e = g \quad \therefore \; 01 = 10 = 11 \\ d = e = g = 0 \quad \therefore \; 00 = 01 = 10 = 11 \end{matrix}$$

It should be noted that $d = 0$, $e = 0$ or $g = 0$ does not in itself lead to equality of phenotypic values. The different cases, apart from $00 = 01 = 10 = 11$, have been inserted in table 3.

Change in environment or selection in populations of genotypes may cause the different types to pass into each other. When by some selection device g is increased, *e.g.* type 4*a'*, changes into 3', 1*a*, 3 and 4*a* respectively. Decrease of d leads from, *e.g.* type 1*a* via 1*b* to 2 or 2', depending on whether $g > 0$ or $g < 0$. When we then allow d to become negative, we obtain $e > -d > |g|$ and finally $-d > e > |g|$. These types derive from 1*b* and 1*a* by interchanging the columns. Having defined d as non-negative, however, the selection result will under the present approach make the particular population change its label G_0 into G_1 or *vice versa*.

Acknowledgments.—I wish to thank Professor K. Mather for helpful discussion and for the opportunity of working in his department. This work was carried out while in receipt of a grant from the Netherlands Organisation for Pure Research (Z.W.O.).

REFERENCES

HALDANE, J. B. S. 1946. The interaction of nature and nurture. *Ann. Eugenics, 13,* 197-205.

MATHER, K., AND MORLEY JONES, R. 1958. Interaction of genotype and environment in continuous variation. I. Descriptions. *Biometrics, 14,* 343-359.

OUTCROSSING ON HOMOSTYLE PRIMROSES

JACK L. CROSBY
Botany Department, Durham Colleges in the University of Durham

1. INTRODUCTION

Bodmer (1958) gives figures which he claims demonstrate that homostyle primroses (*Primula vulgaris*) are cross-fertilised in cultivation to the extent of about 80 per cent., and he considers that this is also true of homostyles in wild populations. My own results from primroses both in the wild and in cultivation lend no support to this view. Indeed, they strongly suggest a much lower rate of cross-fertilisation, as this note will show.

Bodmer's estimate of outcrossing is based on the following observation, made over a number of years. Families grown from seed set by open pollination of heterozygous homostyles were found to have a significant deficiency of pins, when compared with the expectation that the same plants on selfing would have segregated one pin : three homostyles. His parent plants were grown in an open bed in which one third of the homostyles are stated to have been homozygous. He therefore concludes that the shortage of pins arose from cross-pollination, the presence of homozygous homostyles among the potential pollinators producing excess of homostyles among the progeny of the heterozygotes. This attempt to estimate the amount of outcrossing depends directly on the assumption that heterozygous homostyles on selfing always segregate to give 25 per cent. of pins. My own results, however, show that in many cases, possibly in most, there is a clear deficiency of pin plants in families from controlled self-pollination of heterozygous homostyles.

2. ESTIMATION OF OUTCROSSING IN CULTIVATION

A much more direct and accurate way of determining the extent of cross-fertilisation of homostyles under garden conditions is to grow them in a bed containing thrums (which will donate pollen as readily as homostyles in cross-pollination), and to collect their seeds and grow families from them. Every thrum appearing among the progeny is a definite indication of a foreign pollen grain.

In 1948, 54 capsules were taken from 14 homostyles (6 heterozygous, 8 homozygous), growing in a bed containing 203 pins (*ss*), 176 thrums (*Ss*), 31 heterozygous homostyles (*s's*), and 12 homozygous homostyles (*s's'*). The homozygous homostyles were together near one end of the bed ; the other types were distributed more or less evenly through the bed. Table 1 shows the progeny : the rather poor germination was common to almost all families sown at the same time as these. Of the 568 plants produced, only 17 were thrums ; of these 9 came from one capsule and 4 from a second. Since about 40 per cent. of the available high-anther pollen would carry *S*, the 17 thrums represent about 7·5 per cent. of cross-fertilisation of the homostyles. The only alternative possibility would be a high death rate among thrum-homostyle heterozygotes (*Ss'*), but this is contradicted by the fact that of the thrum plants which did appear, all but 2 came from

homozygous homostyles which can produce only *Ss'* thrums ; while of approximately 115 *s* ovules on heterozygotes, not more than 2 were fertilised by *S* grains. Also, a few small families from crosses between thrums and homozygous homostyles actually show an excess of thrums over homostyles. It is clear that any inviability which there may be of *Ss'* zygotes is insufficient to require any large amendment of the estimate of outcrossing.

TABLE 1

Open pollination of homostyles in a bed containing 203 pins, 176 thrums, 31 heterozygous homostyles, and 12 homozygous homostyles.

Plant	Distance from homozygous homostyles, in feet	No. of capsules	Progeny P	T	H
Heterozygous homostyles ; D, E, F are sister plants					
A	16	7	10	0	43
B	12	4	2	0	21
C	7	5	9	[illegible]	40
D	3	4	5	1	18
E	3	3	11	0	25
F	3	[illegible]	14	1	33
		31	51	2	180
Homozygous homostyles ; sister plants					
G		2	...	0	38
H		2	...	1	26
I		[illegible]	...	[illegible]	36
J		2	...	0	30
K		5	...	1	82
L		3	...	9*	38
M		3	...	[illegible]	26
N		4	...	4*	44
		23	...	15	320
	Grand totals .	54	51	17	500

* All from one capsule.

Closer examination of table 1 shows that 3 of the heterozygotes, A, B and C, actually produced a greater shortage of pins (taken together) than all but the first two years of Bodmer's results. The total for A, B, C is 21 pins : 104 homostyles which differs significantly from a 1 : 3 ratio ($\chi^2_{[1]} = 4{\cdot}5$) ; it may be considered separately from the total for D, E, F, since the two totals are probably heterogeneous ($\chi^2_{[1]} = 4{\cdot}1$).

The shortage of pins from A, B, C can hardly have been due to outcrossing by homozygous homostyles, since there were far more thrums than homostyles in the bed, and there is no evidence of outcrossing by thrums onto these 3 plants. Moreover, they were much farther from the homozygous homostyles than D, E, F, which have no deficiency of pins. The possibility that one or two exceptional cross-pollinations by homozygous homostyles may have seriously upset the ratio may be ruled out, since the deficiency of pins was shown by 13 of the 16 capsules. The most likely explanation is that the 3 plants would have shown a similar deficit of pins

on controlled self-pollination, and that the plants D, E and F (which were sister plants) differed from them in this respect.

3. PROGENY FROM CONTROLLED POLLINATIONS

That this deficit of pins is a general phenomenon of F_2 families from heterozygous homostyles is shown by table 2, which gives the results of controlled self-pollinations and of crosses between heterozygous homostyles. The figures include all appropriate families for which data are available, and are grouped according to the experimental series in which they were carried out.

TABLE 2

Controlled pollinations of heterozygous homostyles

Cross or self	No. of plants involved	No. of families	Progeny P	Progeny H
Self	11 sister plants	11	58	195
Self	17 unrelated plants from 11 populations	18	34	131
Cross		4	0	19
Self	1	4	50	155
Self	1	6	5	58
Self	1	5	8	35
Cross	7 unrelated, including previous 3	6	22	72
			177	665

The total shows a significant shortage of pins from the expected 1 : 3 ($\chi^2_{[1]} = 7{\cdot}1$), but the close correspondence with the ratio of Bodmer's total is presumably a coincidence, since the individual series are probably heterogeneous ($\chi^2_{[6]} = 14{\cdot}0$). These results strongly support the suggestion from the open-pollinated families that many heterozygous homostyles show a considerable deficit of pins on selfing, and that there is variability in this respect among different plants.

Bodmer's results are most reasonably explained on this basis. It is true that he allowed some heterozygotes to self, and obtained 130 pins to 390 homostyles. But this ratio is based only on segregating families ; we are not told the size of the families used, so we have no means of estimating how many families from heterozygotes might have been excluded because no pins appeared in them ; the result will, however, be taken at its face value.

These F_2 families were produced in 1956 and 1957, that is after the latest and much the largest of Bodmer's open-pollinated progeny groups had shown not merely no deficiency, but a slight excess of pins. In the absence of specific information in Bodmer's paper, it would seem reasonable to infer that these families are most nearly related to the most recent of his open-pollinated series. That is, as controls they are most relevant to his large 1955 score, with its slight excess of pins, and their relevance decreases as one goes back in time. Yet in carrying out a contingency test, he omits the 1955 plants and tests the F_2 against the doubtfully relevant earlier results.

It seems most likely that the earlier plants had a tendency to an F_2 shortage of pins, and that this tendency decreased until it became slight

by the time the 1955 families and the subsequent F_2 families were produced. Even if we exclude the 1955 results, the others still suggest a trend in this direction.

If Bodmer wishes to establish his interpretation, he must demonstrate that the earlier plants would have segregated 1 : 3 on selfing, and that the deficit of pins in the open-pollinated families was matched by an excess of homozygous homostyles. The latter point is critical ; the only evidence which Bodmer offers is that he found no significant difference from a homozygote : heterozygote ratio of 1 : 2, and he himself assumes this ratio in his calculations. An extensive series of test-crosses is called for. Bodmer makes no real attempt to explain why there should have been no cross-pollination in 1955.

But whatever the validity of Bodmer's results may be, the application of conclusions drawn from cultivated families to natural populations requires the greatest caution. Conditions are different. As a case in point, we may consider his observation that in his plants the stigma is frequently to be seen above the anthers in young flowers. Now plants grown in cultivation normally flower at least two or three weeks before their wild counterparts, when the weather is relatively colder. Under these conditions, and under the usually exposed conditions of the garden bed, the corolla tube (which is usually much more sensitive to ordinary environmental changes than the style) is relatively short, and any tendency of the stigma to project is enhanced. I have noticed this in a number of my own families. This situation has little relevance to natural conditions. I must have examined nearly 50,000 wild homostyle flowers ; a large proportion of these will have been opening buds or newly-opened flowers, since where there is any choice it is these that are taken for examination. It can be stated quite definitely that in newly-opened flowers it is unusual for the stigma to be visible above the anthers, and rare for it to be entirely clear of them.

Bodmer found a delay of some days in anther dehiscence in many of his cultivated plants, but presents no evidence to show that pollinating insects ever visit primroses before the flowers are fully open and the anthers have dehisced. His observations were made in hot sunny weather ; but hot sunny spring days are often accompanied by very cold nights, especially in Cambridge. In my experience of natural populations, delay in anther dehiscence is unusual except in the sort of weather in which very few pollinators are flying.

4. ESTIMATION OF OUTCROSSING IN NATURAL POPULATIONS

One method of estimating the amount of outcrossing on wild homostyles is on the basis that cross-fertilisation by thrums will produce some plants of constitution *Ss'*, that is thrum-homostyle heterozygotes. Such plants should be found in natural populations if homostyles are regularly cross-pollinated. The actual existence of such plants, and their implications, has already been pointed out (Crosby, 1949). Detailed results can now be given.

Test crosses have been made on 95 thrum plants from 17 populations of roughly comparable constitution chosen as likely to show the highest proportion of *Ss'* plants ; the average homostyle content was about 52 per cent. It can be shown that, with 80 per cent. outcrossing, about 20 of the

tested thrums should have been *Ss'*. The actual number was 2 ; the remaining 93 were *Ss*. This direct attempt to estimate outcrossing on homostyles in natural conditions suggests a value lying between 5 and 10 per cent., and this value must carry more weight than Bodmer's indirect and doubtfully valid estimation on cultivated plants descended from a few plants taken originally from a single locality.

Finally, it is difficult to see how the very marked shortage of thrums as compared with pins which is found in populations with many homostyles (Crosby, 1949) can be accounted for other than by supposing thrum pollen to be at a serious disadvantage as compared with homostyle pollen, and this can only result from a high frequency of self-pollination of homostyles. The whole question of pollination and fertilisation of the primrose has been carefully studied by several quite different methods, and an account of this work is in course of preparation. It will include a discussion of the deficit of pins which is found on selfing heterozygous homostyles.

5. SUMMARY

Bodmer's indirect estimate of a high frequency of outcrossing among homostyle primroses (*Primula vulgaris*) is questioned on the following grounds.

Heterozygous homostyles on controlled self-pollination commonly produce less than the expected 25 per cent. of pins ; this conflicts with the basic assumption of Bodmer's calculations, and offers a likely explanation of his results.

The use of thrums as direct indicators of outcrossing has given experimental evidence of about 7·5 per cent. outcrossing of homostyles under garden conditions.

On a similar basis, an estimate of outcrossing on homostyles under natural conditions, involving 17 populations, suggests a value between 5 and 10 per cent.

The known constitutions of populations containing homostyles require for their explanation a considerable competitive disadvantage of thrum pollen, and this can hardly arise except through a high degree of self-fertilisation of homostyles.

6. REFERENCES

BODMER, W. F. 1958. Natural crossing between homostyle plants of *Primula vulgaris*. *Heredity*, *12*, 363-370.

CROSBY, J. L. 1949. Selection of an unfavourable gene-complex. *Evolution*, *3*, 212-230.

REVIEWS

THE STRATEGY OF THE GENES. By C. H. Waddington, with an appendix by H. Kacser. London : George Allen & Unwin Ltd., 1957. ix+262 pp. 28s.

Essay writing is very much part of the British scientific tradition, and those who indulge in it are conscious of the fact that no other kind of writing can take its place. Papers in learned journals, besides being set out in a form which perpetuates an out-of-date conception of the nature of scientific enquiry, are necessarily confined to first-order speculations, *i.e.* to hypotheses which lead to directly verifiable predictions. Reviews show signs of degenerating into annotated bibliographies and are retrospective in their cast of thought ; books are too commodious and monographs too austere. A good essay must be written in a style which is in itself a source of pleasure ; must be imaginative ; must be interesting ; and must also have a certain strong intellectual thrust. Waddington's essays have all these qualities in full measure, and they will be read with intent interest, even by the perhaps unnecessarily large number of people whom they will annoy.

In the " Cybernetics of Development " Waddington attempts to devise, not indeed a theory of development, but a system of concepts in terms of which such a theory might one day be propounded. Development, says Waddington, has three chief ingredients : the " regionalisation " in which different parts of the germ come to be allocated to the development of different organ systems ; histogenesis, in which each such system acquires its distinctive cellular characteristics ; and morphogenesis, the process of taking shape. Developpent is a continuous and gradual process, but its final products vary discontinuously in the sense that the different tissues of the adult animal are sharply distinct, instead of grading into one another. (Waddington says that this is not theoretically inevitable, but it is not clear what he means : if it is a general truth, then one should attempt, as he does himself, to devise a theory from which discontinuity should follow as a logical consequence.)

Discontinuity, then, is the " focal concept " ; the fundamental mechanism of development must therefore be one " by which the different cytoplasms, or oöplasms, which characterise the different regions of the egg, act differentially on the nuclei so as to encourage the activity of certain genes in one region, of other genes in other places ". " What we need to understand . . . is how originally slight differences in the materials available to the genes in different parts of the egg can become exaggerated and produce large and clear-cut differences between the final tissues." Waddington sketches out a possible mathematical model which works in such a way as to exaggerate initial differences and so provide the discontinuity that the facts call for. But (he goes on) there is more in regionalisation than this : the pathway followed in the movement towards some particular distinct end state is a " most favoured path ", in the sense that departures from it are corrected and restored. Development is " canalised ". The concrete model chosen by Waddington to illustrate the progress of development is his well-known " epigenetic landscape ", in which a ball rolling down a tilted, folded surface is confronted with a sequence of alternative pathways, channels or valleys which finally debouch at the lower

edge. The tilt of the landscape, which will vary from place to place, governs the speed of the rolling ball and may be used to illustrate the rate of progress of development; the cross-section of the landscape, at right angles to the path of the ball, represents the "strength of the tendency of the system to return to normality", for if the pathway is broad and shallow the ball can meander more freely than when the pathway is grooved and steep. Waddington argues warmly for a new word to describe a process analogous to that which rights the ball when disturbances have put it off its course: "homeorhesis" is suggested, and "homeostasis" should be confined to the restoration of particular single values to variable quantities like pH, oxygen tension, gene frequency, and the like. The favoured pathway itself is called a "creode". Here, without criticising his terminology ("creode" is a good word, and it will be interesting to see if it sticks) it should be said that Waddington is usually impatient of the terminology devised by others, not making due allowance, it seems to me, for the fact that these other terminologies were devised by people who had somewhat different problems and purposes in mind. Thus the terminology of "polygenes" is an appropriate and useful *genetic* way of describing the factorial basis of continuous variation, and Thoday's "developmental flexibility", though it does refer to two different ways of accomplishing adaptation, will serve perfectly well when, as in the study of evolutionary problems, we are interested in the accomplishment itself rather than in the means by which it has been achieved.

Waddington's conception of the physical mechanisms underlying creodes is, naturally enough, expressed in very hesitant terms. He has been influenced by the discoveries made in the past ten or fifteen years about the degree of organisation, both anatomical and functional, that is to be found in cellular particles in the size range 0·1 μ to 10 μ, *e.g.* in mitochondria; and he is prepared to envisage a developmental mechanism in which such highly organised particles mediate between genes and developmental processes. In the analysis of creodes themselves, Waddington suggests that (in spite of some evidence to the contrary) it might still be useful to distinguish between "focal" and "peripheral" factors in development and to concentrate on the focal factors—those which, like inductor substances, are necessary for the very existence of tissues and organs. The focal factors might depend upon the production of single key substances which "will come to dominate the whole anabolism of the cell".

This first chapter ends rather lamely—which is not to say that anyone else could do better; but that it should indeed do so with the centenary of *Entwicklungsmechanik* lying not so far ahead is symptomatic of a malaise that afflicts the whole of modern analytic embryology. Embryology consists on the one hand of a large number of very beautiful experiments, often complete and decisive in themselves; and on the other hand of a number of "theories of development" among which Waddington's seems to be the most convincingly argued and the best thought out. But between the two is an almost unbridged chasm: the experiments do not seem to sustain the theories in any very particular way, and the theories do not point towards the execution of particular experiments. There simply *is* no theory of development in the sense in which Mendelism is a theory that accounts for the results of breeding experiments. The facts of embryology are still too much like characters in search of an author, or happenings in search of a plot.

The agenda of Waddington's second long essay, " Selection of, for and by ", can be put in his own words : " It would . . . appear that the known processes of selection are powerful and flexible enough to account for . . . observed evolutionary changes, provided that the material they have to work on—the available variability—can be accepted as adequate in kind. It is in connection with the nature of the available variation that modern theories of evolution are most in need of expansion." Waddington goes on to say that three kinds of enquiry seem to be called for. The first is upon the problem of adaptation : " Is it really sufficient to suppose that the extraordinarily precise fitting of an animal into its ecological niche is due solely to the selection of random variations ? " The question is considered at length in the chapter devoted mainly to the phenomenon of genetic assimilation, *i.e.* of the genetic fixation of phenotypic changes originally brought about by the action of the environment. The experimental work upon which the idea of genetic assimilation was based is thoroughly familiar to geneticists and needs no recapitulation here. What is new is this. In most experiments on genetic assimilation (I had assumed, as perhaps others had, in all) there is strong evidence that " the genetic basis for the assimilated genotype was . . . present in the initial population ; while there is little that positively suggests—and that not very compellingly—that the environmental stress has called forth the new variation ". Correspondingly, genetic assimilation will not occur if one starts with inbred stocks. There are, however three cases in which there has arisen the faint suspicion that the assimilated stock contains genes which were *not* present in the initial population, and the correspondingly faint possibility that the environment might have *directed* the occurrence of one or more mutations of specific adaptive value. Waddington does not accept this interpretation, nor yet does he totally reject it ; he thinks it conceivable that the environment should have done so.

The two other lines of enquiry prompted by the agenda set out above can be put as two questions : (*a*) are the genetic differences between larger taxonomic categories of the same kind as the differences between subspecies or related species, and (*b*) are the evolutionary processes that can operate over great lengths of time merely the sum of those which work over short stretches of time ? The orthodox answer to both these questions is Yes, but it is not spoken with complete conviction, and Waddington is prepared to keep an open mind.

" The Organisation of the Gene Pool " is a study of the use of the idea of canalisation in the interpretation of fitness. The attainment of the phenotypic optimum must be brought about by a compromise between two extreme possibilities, (*a*) that any genetic variation which might cause departure from the phenotypic optimum should be eliminated, and (*b*) that development should be so highly canalised or buffered that the same end result should be achieved no matter what the genetic variation may be. Waddington proceeds to discuss the evolution of developmental buffering in terms of the shape of the cross-section of the epigenetic landscape described above. If the cross-section is smoothly U-shaped, small departures from the mean will abound, though large departures will be rare ; if it is sharply V-shaped, then departures from the prescribed genotype will be very difficult to achieve. The cross-section need not be symmetrical : departures in one direction may often be easier than departures in the other direction.

The book ends with an interesting and very well written chapter by H. Kacser on certain life-like properties that are to be seen in the chemical kinetics of open systems. Kacser's treatment of " equifinality " is in some ways more realistic and powerful than, for example, Sommerhoff's treatment, in terms of classical mechanics, in his *Analytical Biology*—an analysis that fully deserves the careful attention which Waddington gives it in an earlier chapter. In closed systems, the equilibrium concentrations of the reagents are independent of the absolute values of the rates of chemical transformation within the system, but do depend upon their initial concentrations. In open systems, systems in which matter enters and leaves, the concentrations of the reagents when a steady state has been achieved are independent of their initial concentrations ; such a system will show " regulatory " behaviour when the initial concentrations are altered and will " regenerate " if some part of the reagents is removed. There are other life-like properties besides. In the main, the attempt to breathe real life into the chemical-kinetic model becomes less convincing as it becomes more detailed, but that such a model should exist at all is a rewarding and reassuring fact. The study of such models by biologists should, as Kacser suggests, inculcate a salutary habit of thought about the complexities of biological processes ; and there is in fact more worth reading in Kacser's fifty pages than in a whole library of *Naturphilosophie*.

There is very much more in Waddington's volume of essays than will appear from this review ; there is, indeed, something worth thinking about on every page. It is a book to be read more often than once or twice.

P. B. MEDAWAR.

THE SANCTITY OF LIFE AND THE CRIMINAL LAW. By Glanville Williams. London : Faber & Faber. 1958. Pp. 319. 30s.

A system of law, we are permitted to imagine, might be derived from a knowledge of life fully informed by the methods of science. It might be made consistent with our understanding of biology. And it might be constructed so as to favour the survival in evolution of any given human society, changing with the changing needs and conditions of that society. The systems of law which the modern western nations inherit and also cherish are not, however, constructed with such ends in view. They are derived from views of the origin and behaviour of man expressed by the Christian Fathers, celibates and saints, and derived by them from the study of their own natures and of certain ancient and sacred writings.

The conflict between these opposed points of view, the inductive point of view of science and the deductive point of view of religion and (as a rule) of the law, is growing as the powers of science are recognised. It is sharpest in what concerns questions, not of property as the Marxist believes, but of birth and death. It is these questions and this conflict which Dr Glanville Williams is considering in this volume of Carpentier Lectures delivered at Columbia University.

Dr Williams divides his discussion between problems which may be arranged in order in the cycle of human life, namely sterilisation, contraception, or, its reciprocal, artificial insemination, abortion, infanticide, suicide, and euthanasia. All these problems, save the last, have a genetic component which gives a unity to the whole, a unity of which the author, being a lawyer, is less aware. Nevertheless his work achieves something

bigger, bolder and probably more effective than has yet been seen. He has brought the religious, the legal and the biological evidence and arguments together and set them before us in a long and broad historical perspective.

One difficulty that Dr Williams meets is that scientific developments, for example in the use of blood groups for determining paternity, are so rapid that the grounds for discussing and deciding moral and legal principles may be shifted in the course of a few years. A graver difficulty is that he sometimes relies for opinions about medical genetics on medical experts. For example, there is the " specialist in the subject of fertility " who wrote " I used to make enquiries of the infertile male about his family but found there was no relationship between the fertility of the parents and that of the children ". This specialist continued " *and I have given up taking family history into account* " (p. 114, my italics).

At some stage in the development of artificial insemination the medical experts will have to be told about the genetic evidence, about what we already know. But many new problems are raised for genetics. If A.I.D. is merely propagating infertility the situation carries its own remedy. But artificial insemination might be used to propagate exceptionally high fertility. What then would be its consequences? But since it certainly is being used to propagate many other special genetic properties, its genetic methods, the use of inbreeding and outbreeding and their effects on marriage and fertility of the first and second generation, these are questions which we still have to examine.

The great difficulty in writing a book of this kind is naturally to avoid smothering the principles in a welter of detail. A thousand references and hundreds of verbatim quotations give weight to each small argument but they also give heaviness to the whole. The text could have been lightened by putting the bibliography and many quotations at the end. The argument could also have been eased by a general summary of principles. Such a summary would point to the general effects of Catholic doctrine; the items of Dr Williams' account are some highly comic, like the theory of the origin of lust (p. 58), but they are all partly tragic; and in every chapter they accumulate and add up to the appalling total of *no, no, no*. Such a summary would also note the utter conflict of method between accepted ideas of causation, the legal idea being explicitly " common sense " and explicitly opposed to the " philosophical or technical or scientific " (Mr Justice Devlin, p. 289). And it would note the dangerously rapid growth of such conflicts between scientific method and discovery on the one hand and the traditional forms and ideas of society on the other.

These conflicts are relieved by the diversity of traditional opinion and religious doctrine and by the consequent diversity of legislation in different countries and (most significantly) in different states of the U.S.A. In this diversity the law itself also plays a part for, as Dr Williams points out, it would be impossible to invent situations better calculated to entangle legal definitions and judgments than those arising, for example, from artificial insemination in man.

The lesson of this book the author would suppose lies in its practical proposals. But for those who teach in universities there is a larger lesson. It is that the scientific study of life, its achievements and prospects, should be more widely taught. It should, in the years of their innocence, be brought to the notice of those who will later be taking it upon themselves, in their

several spheres and with matured authority, to lay down the law for ordinary men and women. C. D. DARLINGTON.

VERERBUNGSLEHRE : Methoden und Resultate. By Arne Müntzing. Translated by D. V. Wettstein. Stuttgart : Gustav Fischer Verlag. 1958. Pp. xi + 303. DM 42.

Professor Müntzing's Swedish work of 1953 has now been translated into German. As a text-book it is calculated to serve the purposes of university teaching. It is arranged in twenty-seven chapters. It covers a wide range of problems and reveals a great breadth of experience.

The method of treatment is historical and practical rather than theoretical and the result has advantages for the student who is beginning. It also has advantages for teachers who find theory difficult. It does, however, lead to some lack of connectedness. For example, continuous variation is a problem connected with many aspects of biology. Müntzing considers the views of Nilsson-Ehle and Mather on it in one place (p. 73), those of Baur in another place (p. 128) and those of Darwin in various places. And the different considerations are not connected. Nor, of course, are they entirely consistent.

Some disconnectedness also appears in the discussion of inbreeding. One old story told here ought not to be repeated any longer. Müntzing says that Cleopatra was the result of repeated (" wiederholter ") brother-sister mating (p. 170). This implies in genetics successive matings with cumulative effect. The Ptolemies, however, or so it is believed, had incest only in alternate generations and the effect, therefore, was not cumulative. It would, however, be more important to explain to the reader the general breeding systems of man rather than to refer to such exceptions. For man (as it now appears) is genetically the most significant of all animals and the results of his breeding are continually before our eyes.

Connectedness is one of the gifts of genetics to biology. Providing a framework and a method is another. In this book we are told (p. 116) that Renner in 1917 has *proved* (bewiesen) that the hybrid species of *Œnothera* were complex heterozygotes. But this kind of statement misses the opportunity of helping the reader to understand scientific method in genetics. What Renner did was to propose a hypothesis, to invent a name for it and to advance evidence in favour of it. All this prodigiously advanced our understanding of genetics. But it did not precisely prove anything. The distinctions between these processes are the most important we learn from the study of genetics. They are the reasons why some kinds of biologists find genetics interesting, others find it difficult, and others again merely useful.

There is one respect in which the author does too little justice to his own book. The illustrations are numerous and many of them are valuable. It is from them that the reader will wish to turn to the original papers, sometimes to extend his study, sometimes to correct it. But there is no means of doing so. The origins quoted are often second hand and never fully stated. Some like Karper (fig. 21) and Böhnert (fig. 119) seem to be *nomina nuda*. This is unfair to the original author as well as the reader. And when percentages do not add up to a hundred (fig. 93) or when magnifications are said to be the same which cannot be the same (fig. 126i) the result disturbs the serious reader.

There are, not one way, but a great variety of ways of writing a book on

genetics that will serve a useful purpose. Although the present work seems to have some shortcomings it will meet a need in a German translation, as indeed it might well do in an English translation also. C. D. D.

GENETISCHES UND CYTOGENETISCHES WÖRTERBUCH. Second Edition. By R. Rieger and A. Michaelis. Berlin : Springer-Verlag. 1958. Pp. 648. DM 49.60.

A dictionary may be designed for the expert or for the student. This work, however, is an encyclopædia rather than a dictionary and will be useful for all stages of study. It is comprehensive and impartial rather than selective and discriminating. This means that there is a great deal of matter of merely historic interest. But as the history of genetics becomes of greater importance the use will increase. It is already worth knowing that Wallace invented " Darwinism " and Punnett " Mendelism ".

The English equivalents of the German terms are usually given but of course the reciprocal is not true. English-speaking students who expect variegation in plants to be mentioned under " variegation " will be disappointed. Again some German words, like " Prävalenz " have never been translated into English since their use is pre-empted by other meanings. It may well be, therefore, that only an English translation of this book could reap the full reward of the work. It is indeed time that something on this level was attempted in English.

The 149 diagrams are mostly clear and helpful, although fig. 26*b* seems to be wrong, and fig. 136 doubtful. No doubt the authors will be grateful for correspondents who point out the omissions or correct the few misspellings, post-datings and other mutations which occur among their 1500 references. Altogether they are to be thanked for their devoted labours.

C. D. D.

NATÜRLICHE UND KÜNSTLICHE ERBÄNDERUNGEN : Probleme der Mutationsforschung. By Hans Marquardt. Hamburg : Rowohlt. 1957. Pp. 177. DM 1.90.

This is an attempt to popularise genetics and its use in understanding natural and induced mutation. The attempt is praiseworthy but the author has not understood certain needs of popularisation.

Fig. 29*a* provides an example of Dr Marquardt's method. It is a diagram, not a very clear diagram, introducing breakage and reunion of chromosomes. It represents not two breaks, however, but three. It shows a broken terminal segment becoming an intercalary segment. But this is something which is not supposed to happen after breakage. And the author describes the process in a compound sentence of seven lines.

Dr Marquardt's text seems to include much that is unnecessary to the argument. It also leaves out the large questions of population genetics, cell physiology and gene evolution without which no conclusion can be drawn. C. D. D.

EVOLUTION OF GENETIC SYSTEMS. Second Edition. By C. D. Darlington. Edinburgh : Oliver and Boyd. 1958. Pp. 265+xii. 21s.

In his preface the author tells us that " This book was first intended to show how helpful genetics could be to other sciences, and also how helpful other sciences could be to genetics. Whether or not the argument was justified the book has proved to be of use in introducing the subject to beginners. I have therefore brought it up to date in a new edition." In so introducing the new edition, Professor Darlington is over-modest. True,

the book has proved of great value in introducing genetics in its broadest sense to the student, and it will go on being of value ; but let us not forget that when the *Evolution of Genetic Systems* first appeared nearly twenty years ago it expounded for the first time the thesis that the genetic materials and genetic structures of species were at once the basis for evolution in the future and the product of evolution in the past. Genetics as a science is far broader, deeper and stronger than it was twenty-five years ago, and none has contributed more than Darlington to making it so. Nearly always his ideas and generalisations, ranging from chromosome mechanics to the interrelations of heredity, development and infection, have been sharply challenged and hotly debated—though usually tacitly accepted in the end. Basic as it is, however, his notion of the genetic system and its evolution seems never to have aroused the same argument : it has been quietly adopted and built into the pattern of genetics as we see it to-day. Such is the tribute paid to the first edition of this book, which has indeed been described as the greatest among the many great contributions its author has made to genetics. And such is the background against which we welcome the second edition.

This new edition is described as " revised and enlarged ", and so it is. New chapters have been added on " The Management of the Cell ", " Types of Plasmagene ", " Heredity and Infection " and " Initiative in Evolution ". These deal essentially with new aspects that have appeared in genetics since the first edition was written—with the co-operation and integration of nucleus and cytoplasm, with cytoplasmic particles, with viruses and virus-gene relations as we see them in bacteria, and with broader concepts of evolution as genetics show them to us. The older chapters too have been extensively re-written to incorporate the mass of new material that has become available in relation both to cell structures and population behaviour during the past twenty years, though unfortunately not all the consequent changes of detail, in for example the figure references, have been caught. The new edition indeed gives us the up-to-date picture as Darlington sees it, yet the change is one of development rather than of reconstruction. The fundamental ideas remain the same, for time and growth have required no change of them. To those who learned from the earlier version it will be a pleasure as well as an interest to see how the newer development is assimilated to the older structure. To the student, the new edition will offer the same fascination and profit that its predecessor did to his forerunner of nearly a generation ago. KENNETH MATHER.

BIBLIOGRAPHY ON THE GENETICS OF *DROSOPHILA*. Part 3. By I. H. Herskowitz. Indiana University Publications, Science Series No. 21. Bloomington : Indiana University Press. 1958. Pp. 296+ix. $2.50.

This new part of the *Bibliography*, though produced by a different publisher, follows much the same style as did Part 2 (reviewed in *Heredity*, *8*, 289-290). It includes rather more than 3100 references covering the years 1951 to 1956 inclusive, and as before these are arranged alphabetically by their senior authors, with full cross-references against the junior authors. The Index is now in three parts, as a Geographical listing is added to the pre-existing General and Systematic sections. This new addition to the *Bibliography on the Genetics of* Drosophila will be welcomed by the many who must have found its predecessors so useful. KENNETH MATHER.

GENETICAL SOCIETY OF GREAT BRITAIN

ABSTRACTS of Papers read at the HUNDRED AND TWENTY-EIGHTH MEETING of the Society, held on 14th and 15th November 1958, at UNIVERSITY COLLEGE, LONDON

ABH SECRETOR AND LEWIS CHARACTERS IN MAN

S. D. LAWLER and R. MARSHALL
Galton Laboratory, University College, London

Using material supplied by Drs I. Bianco, E. Silvestroni and M. Siniscalco, from 320 individuals, including members of 66 families, in Ferrara, Italy, further evidence has been obtained of the interaction of ABH secretor (i.e. *Se, se* alleles) and Lewis (i.e. *L, l* alleles). The ABH secretor status of each individual was determined by standard inhibition tests on saliva.

The presence of Le^a substance in the saliva sample was also determined by inhibition tests. Using an arbitrary scale, each individual was given an inhibition score. In a few cases there was difficulty in classifying individuals as Lewis-positive (*LL* or *Ll*) or Lewis-negative (*ll*). The discrimination was improved by doing similar inhibition tests with anti-Le^b on a selected sample and deriving a total score for the amount of Le^a and Le^b substances. The frequency of *ll* in the unrelated individuals of the whole sample was 10·61 per cent.

Family data supported the hypothesis that *L* (Lewis present) is dominant to *l* (Lewis absent). The presence of Lewis substances in the saliva is independent of the presence of ABH substances, but the amount of Lewis substances in saliva and their presence in detectable amounts on erythrocytes is influenced by the secretor phenotype.

FAMILIAL LOW PSEUDOCHOLINESTERASE LEVEL IN PLASMA

H. LEHMANN, V. PATSTON and E. RYAN
St Bartholomew's Hospital, London

Plasma pseudocholinesterase is measured principally for two practical reasons. The enzyme level is low in liver disease and its determination is used as a liver function test. The short-term action of the widely used relaxant succinyl-dicholine is due to its rapid destruction by pseudocholinesterase. Occasionally a prolonged apnœa—due to excessively prolonged paralysis of the respiratory muscles—is encountered by anæsthetists using succinyl-dicholine, and it can then be traced to a low pseudocholinesterase level. Sometimes this can be explained by liver disease. On occasions no such explanation can be found. This " idiopathic " enzyme deficiency could be shown to be familial. The range of normal enzyme level is wide. Nevertheless with the increasing number of family trees studied a pattern representing the homozygous and heterozygous states can be recognised.

GENETIC CHANGE IN DIPLOID YEAST

E. A. BEVAN
Botany School, Oxford

The frequency of spontaneous change from heterozygosity to homozygosity at specific loci in diploid cells of *Saccharomyces cereviseæ* has been determined using a visible gene marker, namely red colouration. When colonies derived by plating

out white diploid cells of constitution ∝ ad_1 (red) *me tr* +/*a* + (white) + + *ur* are examined, up to 42 per cent. are sectored red and approximately 0·05 per cent. are pure red. All single-cell isolates from these colonies are diploid. Further analyses of single-cell isolates from each of the red and white portions of 55 sectored colonies revealed among the white cells two homozygous methionine-requiring strains and two uracil requirers, and among the red cells one uracil and one methionine requirer.

Such frequencies of genetic change at specific loci are in sharp contrast to those which occur in haploid cells; among 2117 colonies derived from haploid cells *treated* with ultra-violet irradiation and tested for auxotrophy by the " total isolation " technique, only one isolate was found to have mutated at the + /ad_1 locus. In the same experiment nine methionine, six tryptophane and two uracil-requiring auxotrophs were recovered which have not yet been tested for allelism; several loci may therefore be represented by each frequency.

In view of this contrast it is tempting to deduce that a mechanism other than what may be termed " classical " gene mutation is responsible for the high frequency of change at specific loci in heterozygous diploid cells. Alternative explanations to account for these preliminary observations are discussed.

SELF-FERTILITY IN THE FIELD BEAN (*VICIA FABA* L.)

D. G. ROWLANDS
Welsh Plant Breeding Station, Aberystwyth

Estimates of natural cross-pollination in commercial crops of field beans vary from 20-70 per cent., although under normal conditions an average of 30 per cent. is most common. Despite this, few seeds are set when insects are excluded and even after artificial tripping of the flowers is performed complete seed set is rarely attained.

Spontaneous self-fertility (without flower tripping) in a population shows a continuous distribution when measured in terms of total number of seeds produced per plant.

A diallel cross performed using six parent plants to assess their relative cross- and self-compatibilities did not demonstrate consistent superiority of crossing over selfing but rather that certain plants crossed more successfully than others. The progeny from this diallel cross when grown in an insect-proof greenhouse exhibited considerable differences in spontaneous self-fertility. Crosses were generally more self-fertile than selfs and some parents gave progeny which were more self-fertile than others. The Covariance/Variance (Wr/Vr) graph which could be constructed from the data indicated considerable interaction ($b = 0{\cdot}32 \pm 0{\cdot}13$) and this interaction disappeared on the removal of one parental array.

From the data presented, a system of self-incompatibility has been postulated which is largely based on polygenes, and which has developed by the breakdown of a once efficient system through the effect of unconscious selection for uniformity throughout the past history of the crop.

SYNDROME OF DEAFNESS AND GOITRE

G. R. FRASER
Galton Laboratory, University College, London

In recent years several types of familial goitre have been described; they are apparently due to inborn errors of metabolism and are inherited in a recessive manner. One such type is characterised by a failure of the thyroid to bind iodine normally and is also invariably accompanied by severe congenital nerve deafness. Clinical and genetical aspects of this syndrome in several families are discussed.

MATERNAL EFFECT OF *ma-l*$^+$ ON XANTHINE DEHYDROGENASE OF *DROSOPHILA MELANOGASTER*

E. GLASSMAN and H. K. MITCHELL
Institute of Animal Genetics, Edinburgh, and California Institute of Technology, Pasadena, California, U.S.A.

Previous work established that maroon-like (*ma-l*) and rosy (*ry*) eye-colour mutants of *Drosophila melanogaster* are deficient in xanthine dehydrogenase, and therefore the substrates of this enzyme (hypoxanthine and 2-amino-4-hydroxypteridine) accumulate, while the products (uric acid and isoxanthopterin, respectively) are lacking. Recent studies have shown that *ma-l*/*ma-l*$^+$ females exert a maternal effect, in that their *ma-l* progeny have a wild-type eye-colour. However, *ry*$^+$/*ry* females do *not* exert a similar effect on their *ry* progeny. Biochemical studies have shown that in addition to increased amounts of red eye-pigments, maternally-affected *ma-l* flies have traces of isoxanthopterin and uric acid (the enzyme reaction products), as well as traces of the enzyme itself.

The maternal effect is probably due to a substance which *ma-l*$^+$/*ma-l* females pass through the egg, and which is utilised by their *ma-l* progeny. On the other hand, *ma-l*$^+$/*ma-l* ; *ry*/*ry* females still maternally affect *ma-l* progeny, indicating that *ry* synthesizes this compound ; its utilisation is probably blocked in this mutant and thus there is no maternal effect upon *ry*. This suggests that a sequential relationship exists between reactions blocked by these mutants.

THE AXES OF LAMPBRUSH CHROMOSOMES AND OF THEIR LATERAL LOOPS

H. G. CALLAN and H. C. MACGREGOR
Department of Natural History, The University, St Andrews

The structures which project laterally from the axes of newt lampbrush chromosomes have diverse morphologies. Most of these structures are loops, though in some the loop form is obscured by fusion. There is an axis within each lateral loop, and this axis is coated with matrix. Digestion experiments with proteolytic enzymes and nucleases have shown that loop matrices consist of ribonucleoprotein, whereas loop axes, and also the connections between successive loops, contain deoxyribonucleic acid. The linear integrity of a lampbrush chromosome is maintained by an uninterrupted fibre, often centimetres long, of this latter substance.

PHOTOPERIODIC CONTROL OF BREEDING SYSTEM IN *ROTTBŒLLIA EXALTATA*

J. HESLOP-HARRISON
Department of Botany, Queen's University, Belfast

In the short-day grass, *Rottbœllia exaltata* L.f., each segment of the inflorescence bears three flowers, one sessile and hermaphrodite, one sessile and potentially male, and the third, stalked, also potentially male. In daylengths and night temperatures promoting early flowering, the two male flowers are sterile and the anthers of the hermaphrodite flower dehisce before exsertion, so that self-pollination is obligate. If plants are exposed for some weeks after germination to daylengths greater than the critical maximum for flowering and then subsequently induced to flower by transfer to short days, fertility is restored to some of the male flowers, and the anthers of the hermaphrodite flowers are exserted before dehiscence. Under these conditions plants are cross-pollinated. Breeding behaviour thus depends upon the photoperiodic experience of the individual plants, and this may be determined by the time

of the year when germination happens to take place. This environmentally regulated versatility of the breeding system in *Rottbœllia* may be compared with that of *Silene pendula*, a long-day species in which the sexual function of individuals is determined by their photoperiodic experience in early life.

FORWARD AND BACK MUTATION AT THE *PYR*$_3$ LOCUS OF *NEUROSPORA*

J. L. REISSIG
Institute of Animal Genetics, Edinburgh

The citrulline requirement of mutant 33442 (*cit*) is suppressed by mutation of *pyr*$_3$$^+$ to *pyr*$_3$. Strains of the *cit*, *pyr*$_3$$^+$ genotype require only citrulline, while *cit*, *pyr*$_3$ strains require only pyrimidine for growth. This makes it possible to select for forward as well as for back mutants at the *pyr*$_3$ locus.

For forward mutation, a *cit*, *pyr*$_3$$^+$ microconidial strain is plated on minimal supplemented with hydrolysed RNA (plus lysine and canavanine to eliminate leakage). A rate of 3×10^{-5} colonies per viable conidium was obtained after UV treatment (85 per cent. killing). Out of 19 colonies tested, 11 could grow on minimal medium, presumably due to back mutation at the *cit* locus. The rest were pyrimidine-dependent due to forward mutation in the *pyr*$_3$ region. These were tested for the ability to complement each other in heterokaryons, *i.e.* to dispense with the pyrimidine requirement when grown together in pairs. This revealed two complementary groups, with four mutants in each. No recombinants were obtained among $5{\cdot}8 \times 10^4$ ascospores from inter-group crosses.

For back mutations, a *cit*, *pyr*$_3$ strain is plated on minimal plus arginine (or citrulline). Treatment with UV (75 per cent. killing) yielded 10^{-5} colonies per viable microconidium. Roughly half of these were citrulline-dependent, and presumably back mutations at *pyr*$_3$ as shown for one case. The other half could grow on minimal. In one case analysed, this was due to mutation at an unlinked suppressor locus.